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**APPLICATION NUMBER:** *60/541,565*
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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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## INVENTOR(S)

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| Guido | Grandi | Milano, Italy |

*Additional inventors are being named on the* second *separately numbered sheets attached hereto*

## TITLE OF THE INVENTION (500 characters max)

Immunogenic Compositions For Streptococcus Pyogenes

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

☑ Customer Number: 27476

*OR*

| | | | | | |
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| City | | State | | Zip | |
| Country | | Telephone | | Fax | |

## ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 60
☑ Drawing(s) *Number of Sheets* 2
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☐ Other (specify)

## METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.
☑ A check or money order is enclosed to cover the filing fees.
☑ The Director is herby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 03-1664
☐ Payment by credit card. Form PTO-2038 is attached.

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are:



*Respectfully submitted,*

SIGNATURE Rebecca M. Hale

Date Feb 3, 2004

REGISTRATION NO. 45,680 *(if appropriate)*

Docket Number: 20663.002

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***PROVISIONAL APPLICATION COVER SHEET***
***Additional Page***

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Docket Number 20563.002

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Number 2 of 2

# IMMUNOGENIC COMPOSITIONS FOR *STREPTOCOCCUS PYOGENES*

This application incorporates by reference in its entirety U.S. provisional patent application No. 60/491,822, filed on July 31, 2003.

## TECHNICAL FIELD

This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens derived from *Streptococcus pyogenes* and their use in immunisation. All documents cited herein are incorporated by reference in their entirety.

## BACKGROUND ART

Group A streptococcus ("GAS", *S.pyogenes*) is a frequent human pathogen, estimated to be present in between 5-15% of normal individuals without signs of disease. When host defences are compromised, or when the organism is able to exert its virulence, or when it is introduced to vulnerable tissues or hosts, however, an acute infection occurs. Related diseases include puerperal fever, scarlet fever, erysipelas, pharyngitis, impetigo, necrotising fasciitis, myositis and streptococcal toxic shock syndrome.

Although *S.pyogenes* may be treated using antibiotics, a prophylactic vaccine to prevent the onset of disease is desired. Efforts to develop such a vaccine have been ongoing for many decades. While various GAS vaccine approaches have been suggested and some approaches are currently in clinical trials, to date, there are no GAS vaccines available to the public.

It is an object of the invention to provide further and improved compositions for providing immunity against GAS disease and/or infection. The compositions are based on a combination of two or more (*e.g.* three or more) GAS antigens.

## DISCLOSURE OF THE INVENTION

Applicants have discovered a group of thirty GAS antigens that are particularly suitable for immunisation purposes, particularly when used in combinations. In addition, Applicants have identified a GAS antigen (GAS 40) which is particularly immunogenic used either alone or in combinations with additional GAS antigens.

The invention therefore provides an immunogenic composition comprising GAS 40, a fragment thereof or a polypeptide having sequence identity thereto. The invention further includes an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to ten GAS antigens, wherein said combination includes GAS 40 or a fragment thereof or a polypeptide having sequence identity thereto. Preferably, the combination consists of three, four, five, six, or seven GAS antigens. Still more preferably, the combination consists of three, four, or five GAS antigens.

The invention also provides an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of a first antigen group, said

first antigen group consisting of: GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS 202, and GAS 057. These antigens are referred to herein as the 'first antigen group'. Preferably, the combination of GAS antigens consists of three, four, five, six, seven, eight, nine, or ten GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens consists of three, four, or five GAS antigens selected from the first antigen group.

GAS 39, GAS 40, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511 are particularly preferred GAS antigens. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination includes GAS 40.

Representative examples of some of these antigen combinations are discussed below.

The combination of GAS antigens may consist of three GAS antigens selected from the first antigen group. Accordingly, in one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and a third GAS antigen selected from the first antigen group. Preferred combinations include GAS 40, GAS 117 and a third GAS antigen selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and two additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and two GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In another embodiment, the combination of GAS antigens consists of GAS 117 and two additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of four GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and two additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40, GAS 117, and two GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and three additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and three additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In one embodiment, the combination of GAS antigens consists of GAS 117 and three additional antigens selected from the first antigen group.

The combination of GAS antigens may consist of five GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and three additional GAS antigens selected from the first antigen group. Preferred combinations

include GAS 40, GAS 117 and three additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and four additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and four additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In one embodiment, the combination of GAS antigens consists of GAS 117 and four additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of eight GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and six additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40 and seven additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 117 and seven additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of ten GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and eight additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40 and nine additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 117 and nine additional GAS antigens selected from the first antigen group.

Each of the GAS antigens of the first antigen group are described in more detail below. Genomic sequences of at least three GAS strains are publicly available. The genomic sequence of an M1 GAS strain is reported at Ref. 1. The genomic sequence of an M3 GAS strain is reported at Ref. 2. The genomic sequence of an M18 GAS strain is reported at Ref. 3. Preferably, the GAS antigens of the invention comprise polynucleotide or amino acid sequence of an M1, M3 or M18 GAS strains. More preferably, the GAS antigens of the invention comprise a polynucleotide or amino acid sequence of an M1 strain.

*(1) GAS 117*

GAS 117 corresponds to M1 GenBank accession numbers GI:13621679 and GI:15674571, to M3 GenBank accession number GI:21909852, to M18 GenBank accession number GI: 19745578, and is also referred to as 'Spy0448' (M1), 'SpyM3_0316' (M3), and 'SpyM18_0491' (M18). Examples of amino acid and polynucleotide sequences of GAS 117 of an M1 strain are set forth below:

**SEQ ID NO: 1**

MTLKKHYYLLSLLALVTVGAAFNTSQSVSAQVYSNEGYHQHLTDEKSHLQYSKDNAQLQLRNILDGYQND
LGRHYSSYYYYNLRTVMGLSSEQDIEKHYEELKNKLHDMYNHY

**SEQ ID NO: 2**

ATGACACTAAAAAAACACTATTATCTTCTCAGCCTGCTAGCTCTTGTAACGGTTGGTGCTGCCTTTAACA

CAAGCCAGAGTGTCAGTGCACAAGTTTATAGCAATGAAGGGTATCACCAGCATTTGACTGATGAAAAATC
ACACCTGCAATATAGTAAAGACAACGCACAACTTCAATTGAGAAATATCCTTGACGGCTACCAAAATGAC
CTAGGGAGACACTACTCTAGCTATTATTACTACAACCTAAGAACCGTTATGGGACTATCAAGTGAGCAAG
ACATTGAAAAACACTATGAAGAGCTTAAGAACAAGTTACATGATATGTACAATCATTATTAA

Preferred GAS 117 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 1; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 1, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 117 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 1. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 1. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 1. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 1 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide; of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(2) GAS 130*

GAS 130 corresponds to M1 GenBank accession numbers GI:13621794 and GI:15674677, to M3 GenBank accession number GI: 21909954, to M18 GenBank accession number GI: 19745704, and is also referred to as 'Spy0591' (M1), 'SpyM3_0418' (M3), and 'SpyM18_0660' (M18). GAS 130 has potentially been identified as a putative protease. Examples of amino acid and polynucleotide sequences of GAS 130 of an M1 strain are set forth below:

**SEQ ID NO: 3**

MSHMKKRPEVLSPAGTLEKLKVAIDYGADAVFVGGQAYGLRSRAGNFSMEELQEGIDYAHARGAKVYVAA
NMVTHEGNEIGAGEWFRQLRDMGLDAVIVSDPALIVICSTEAPGLEIHLSTQASSTNYETFEFWKAMGLT
RVVLAREVNMAELAEIRKRTDVEIEAFVHGAMCISYSGRCVLSNHMSHRDANRGGCSQSCRWKYDLYDMP
FGGERRSLKGEIPEDYSMSSVDMCMIDHIPDLIENGVDSLKIEGRMKSIHYVSTVTNCYKAAVGAYMESP
EAFYAIKEELIDELWKVAQRELATGFYYGIPTENEQLFGARRKIPQYKFVGEVVAFDSASMTATIRQRNV
IMEGDRIECYGPGFRHFETVVKDLHDADGQKIDRAPNPMELLTISLPREVKPGDMIRACKEGLVNLYQKD
GTSKTVRT

**SEQ ID NO: 4**

ATGTCACATATGAAAAAACGTCCCGAGGTCTTATCACCTGCTGGAACACTTGAAAAATTAAAAGTTGCGA
TTGACTATGGCGCAGATGCTGTTTTTGTTGGAGGGCAGGCCTATGGCCTAAGAAGCCGCGCTGGTAACTT
CTCTATGGAAGAATTGCAAGAAGGCATTGATTATGCACATGCGCGTGGAGCTAAGGTCTATGTTGCTGCT
AACATGGTTACCCACGAAGGGAACGAAATTGGTGCGGGCGAGTGGTTTCGTCAACTGCGTGATATGGGGC
TTGATGCGGTCATTGTTTCAGATCCAGCCTTGATTGTTATTTGTTCAACAGAAGCCCCAGGTTTGGAAAT
TCATTTGTCAACGCAAGCTTCATCTACCAATTACGAGACCTTTGAATTTTGGAAAGCCATGGGCTTGACC
CGAGTTGTTTTAGCTCGCGAGGTTAATATGGCCGAGTTAGCAGAAATCCGCAAGCGGACAGATGTGGAAA
TTGAAGCCTTTGTCCATGGAGCCATGTGTATCTCTTATTCAGGCCGCTGTGTTTTGTCAAACCACATGAG
TCACCGTGATGCCAACAGGGGCGGCTGCTCACAGTCTTGCCGCTGGAAGTATGATTTGTATGACATGCCA
TTTGGAGGAGAGCGCCGCTCCTTAAAAGGGGAAATTCCAGAAGACTATTCTATGTCCTCTGTTGACATGT
GTATGATTGACCATATTCCTGACCTGATTGAAAATGGGGTTGATAGCTTAAAAATTGAAGGCCGAATGAA
ATCTATCCACTACGTCTCAACCGTAACCAACTGTTACAAGGCGGCTGTAGGTGCTTACATGGAAAGCCCA
GAAGCTTTTTATGCTATCAAAGAGGAATTGATTGACGAGTTGTGGAAGGTTGCCCAGCGCGAGTTGGCTA
CAGGTTTTTACTATGGTATCCCAACTGAAAATGAACAATTATTTGGTGCTCGCCGCAAAATTCCACAATA
TAAATTTGTCGGAGAAGTAGTTGCCTTTGACTCAGCTAGCATGACAGCGACCATTCGTCAGCGTAATGTC
ATCATGGAAGGCGATCGGATTGAATGTTATGGACCAGGTTTCCGTCATTTTGAAACGGTTGTTAAGGACT

TACATGATGCGGATGGCCAAAAGATTGACCGTGCCCCAAATCCAATGGAACTCTTAACCATCTCTTTACC
GAGAGAAGTTAAGCCAGGGGATATGATTAGGGCTTGCAAGGAAGGTCTGGTTAACCTCTATCAAAAAGAT
GGCACCAGTAAAACTGTTAGAACATAG

Preferred GAS 130 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 3; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 3, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 130 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 3. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 3. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 3. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(3) GAS 277*

GAS 277 corresponds to M1 GenBank accession numbers GI:13622962 and GI:15675742, to M3 GenBank accession number GI: 21911206, to M18 GenBank accession number GI: 19746852, and is also referred to as 'Spy1939' (M1), 'SpyM3_1670' (M3), and 'SpyM18_2006' (M18). Amino acid and polynucleotide sequences of GAS 277 of an M1 strain are set forth below:

**SEQ ID NO: 5**

MTTMQKTISLLSLALLIGLLGTSGKAISVYAQDQHTDNVIAESTISQVSVEASMRGTEPYIDATVTTDQP
VRQPTQATITLKDASDNTINSWVYTMAAQQRRFTAWFDLTGQKSGDYHVTVTVHTQEKAVTGQSGTVHFD
QNKARKTPTNMQQKDTSKAMTNSVDVDTKAQTNQSANQEIDSTSNPFRSATNHRSTSLKRSTKNEKLTPT
ASNSQKNGSNKTKMLVDKEEVKPTSKRGFPWVLLGLVVSLAAGLFIAIQKVSRRK

**SEQ ID NO: 6**

ATGACAACTATGCAAAAAACAATTAGCTTATTATCACTAGCTTTACTTATTGGTTTGCTGGGGACTTCTG
GCAAAGCCATATCTGTGTATGCACAAGATCAGCACACTGATAATGTTATAGCTGAATCAACTATTAGTCA
GGTCAGTGTTGAAGCCAGTATGCGTGGAACAGAACCTTATATTGATGCTACAGTCACCACAGATCAACCT
GTCAGACAACCAACTCAGGCAACGATAACACTTAAAGACGCTAGTGATAATACTATTAATAGTTGGGTAT
ATACTATGGCAGCGCAACAGCGTCGTTTTACAGCTTGGTTTGATTTAACTGGACAAAAGAGTGGTGACTA
TCATGTAACTGTCACCGTTCATACTCAAGAAAAGGCAGTAACTGGTCAATCAGGAACTGTTCATTTTGAT
CAAAACAAAGCTAGAAAAACACCAACTAATATGCAACAAAAGGATACTTCTAAAGCAATGACGAATTCAG
TCGATGTAGACACAAAAGCTCAAACAAATCAATCAGCTAACCAAGAAATAGATTCTACTTCAAATCCTTT
CAGATCAGCTACTAATCATCGATCAACTTCCTTAAAGCGATCTACTAAAAATGAGAAACTTACACCAACT
GCTAGTAATAGCCAAAAAAACGGTAGCAACAAGACAAAAATGCTAGTGGACAAAGAGGAAGTAAAACCTA
CTTCAAAAAGAGGATTCCCTTGGGTCTTATTAGGTCTAGTAGTCAGTTTAGCTGCAGGTTTATTTATAGC
TATTCAAAAAGTATCTAGACGAAAATAA

Preferred GAS 277 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 5; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 5, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 277 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 5. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 5. Other preferred fragments lack one or more amino acids

(e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 5. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 5 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(4) GAS 236*

GAS 236 corresponds to M1 GenBank accession numbers GI:13622264 and GI:15675106, M3 GenBank accession number GI: 21910321, and to M18 GenBank accession number GI: 19746075, and is also referred to as 'Spy1126' (M1), 'SpyM3_0785' (M3), and 'SpyM18_1087' (M18). Amino acid and polynucleotide sequences of GAS 236 from an M1 strain are set forth below:

**SEQ ID NO: 7**

MTQMNYTGKVKRVAIIANGKYQSKRVASKLFSVFKDDPDFYLSKKNPDIVISIGGDGMLLSAFHMYEKEL
DKVRFVGIHTGHLGFYTDYRDFEVDKLIDNLRKDKGEQISYPILKVAITLDDGRVVKARALNEATVKRIE
KIMVADVIINHVKFESFRGDGISVSTPTGSTAYNKSLGGAVLHPTIEALQLTEISSLNNRVFRTLGSSII
IPKKDKIELVPKRLGIYTISIDNKTYQLKNVTKVEYFIDDEKIHFVSSPSHTSFWERVKDAFIGEIDS

**SEQ ID NO: 8**

ATGACACAGATGAATTATACAGGTAAGGTAAAACGAGTTGCTATTATTGCAAATGGTAAGTACCAAAGTA
AACGCGTCGCCTCCAAACTTTTCTCCGTATTTAAAGATGATCCTGATTTCTATCTTTCAAAGAAAAATCC
GGATATTGTGATTTCTATTGGCGGAGATGGGATGCTCTTATCTGCCTTTCACATGTATGAAAAAGAATTA
GATAAGGTACGTTTTGTAGGAATCCACACCGGTCATCTTGGCTTTTATACCGATTATAGGGATTTTGAAG
TTGATAAATTAATTGATAATTTAAGAAAAGACAAGGGAGAACAAATCTCTTATCCGATTTTAAAAGTTGC
TATTACTTTAGATGATGGTCGTGTGGTTAAAGCGCGTGCTTTGAATGAAGCGACGGTTAAGCGTATTGAA
AAAACGATGGTAGCAGATGTTATTATTAACCATGTCAAATTTGAAAGCTTCCGAGGTGATGGGATTTCAG
TATCGACCCCGACAGGGAGCACAGCCTACAATAAATCTTTAGGTGGTGCTGTCTTGCATCCGACGATTGA
AGCGCTGCAATTGACGGAAATTTCCAGTCTTAATAACCGTGTCTTTAGAACCTTGGGCTCATCAATCATT
ATTCCCAAAAAAGATAAGATTGAGTTAGTGCCAAAACGATTAGGAATTTATACCATTTCCATTGATAATA
AAACCTATCAGTTAAAAAATGTGACGAAGGTGGAGTATTTTATCGACGATGAGAAAATTCATTTTGTTTC
CTCTCCGAGTCATACGAGCTTTTGGGAAAGGGTCAAGGATGCCTTTATTGGAGAGATTGACTCATGA

Preferred GAS 236 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 7; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 7, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 236 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 7. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 7. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 7. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 7 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(5) GAS 040*

GAS 040 corresponds to M1 GenBank accession numbers GI:13621545 and GI:15674449, to M3 GenBank accession number GI: 21909733, to M18 GenBank accession number GI:19745402, and is

also referred to as 'Spy0269' (M1), 'SpyM3_0197' (M3), 'SpyM18_0256' (M18) and 'prgA'. GAS 040 has also been identified as a putative surface exclusion protein. Amino acid and polynucleotide sequences of GAS 040 from an M1 strain are set forth below:

**SEQ ID NO: 9**

MDLEQTKPNQVKQKIALTSTIALLSASVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAV
EKTLSQQKAELTELATALTKTTAEINHLKEQQDNEQKALTSAQEIYTNTLASSEETLLAQGAEHQRELTA
TETELHNAQADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNIAKLNAMISNPDAITKAAQTANDN
TKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQGY
PLEELKKLEASGYIGSASYNNYYKEHADQIIAKASPGNQLNQYQDIPADRNRFVDPDNLTPEVQNELAQF
AAHMINSVRRQLGLPPVTVTAGSQEFARLLSTSYKKTHGNTRPSFVYGQPGVSGHYGVGPHDKTIIEDSA
GASGLIRNDDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYGHAINFLRVDKHNPNAPVYL
GFSTSNVGSLNEHFVMFPESNIANHQRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGKVSSLENRLSAI
HQEADIMAAQAKVSQLQGKLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLA
SLKAALHQTEALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSSLLNAQEA
LAALQAKQSSLEATIATTEHQLTLLKTLANEKEYRHLDEDIATVPDLQVAPPLTGVKPLSYSKIDTTPLV
QEMVKETKQLLEASARLAAENTSLVAEALVGQTSEMVASNAIVSKITSSITQPSSKTSYGSGSSTTSNLI
SDVDESTQRALKAGVVMLAAVGLTGFRFRKESK

**SEQ ID NO: 10**

ATGGACTTAGAACAAACGAAGCCAAACCAAGTTAAGCAGAAAATTGCTTTAACCTCAACAATTGCTTTAT
TGAGTGCCAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGGCGAG
TAATACTCACGACGATAGTTTACCAAAACCAGAAACAATTCAAGAGGCAAAGGCAACTATTGATGCAGTT
GAAAAAACTCTCAGTCAACAAAAAGCAGAACTGACAGAGCTTGCTACCGCTCTGACAAAAACTACTGCTG
AAATCAACCACTTAAAAGAGCAGCAAGATAATGAACAAAAAGCTTTAACCTCTGCACAAGAAATTTACAC
TAATACTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCT
ACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAAGAGACTGCATTGTCAGAACAAAAAG
CTAGCATTTCAGCAGAAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAAAACGTCTGAACAAAATAT
TGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACTAAAGCAGCTCAAACGGCTAATGATAAT
ACAAAAGCATTAAGCTCAGAATTGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAAAGCTAAAGTTAAAA
AGCAATTGACTGAAGAGTTGGCAGCTCAGAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTCGTCT
TAAATCCTCAGCTCCGTCTACTCAAGATAGCATTGTGGGTAATAATACCATGAAAGCACCGCAAGGCTAT
CCTCTTGAAGAACTTAAAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACA
AAGAGCATGCAGATCAAATTATTGCCAAAGCTAGTCCAGGTAATCAATTAAATCAATACCAAGATATTCC
AGCAGATCGTAATCGCTTTGTTGATCCCGATAATTTGACACCAGAAGTGCAAAATGAGCTAGCGCAGTTT
GCAGCTCACATGATTAATAGTGTAAGAAGACAATTAGGTCTACCACCAGTTACTGTTACAGCAGGATCAC
AAGAATTTGCAAGATTACTTAGTACCAGCTATAAGAAAACTCATGGTAATACAAGACCATCATTTGTCTA
CGGACAGCCAGGGGTATCAGGGCATTATGGTGTTGGGCCTCATGATAAAACTATTATTGAAGACTCTGCC
GGAGCGTCAGGGCTCATTCGAAATGATGATAACATGTACGAGAATATCGGTGCTTTTAACGATGTGCATA
CTGTGAATGGTATTAAACGTGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGG
AAATACATACGGCCATGCTATTAACTTTTTACGTGTAGATAAACATAACCCTAATGCGCCTGTTTACCTT
GGATTTTCAACCAGCAATGTAGGATCTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATTGCTA
ACCATCAACGCTTTAATAAGACCCCTATAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGG
CACTGTATCTGATACTATTGCAGCGATCAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTCGGCTATT
CATCAAGAAGCTGATATTATGGCAGCCCAAGCTAAAGTAAGTCAACTTCAAGGTAAATTAGCAAGCACAC
TTAAGCAGTCAGACAGCTTAAATCTCCAAGTGAGACAATTAAATGATACTAAAGGTTCTTTGAGAACAGA
ATTACTAGCAGCTAAAGCAAAACAAGCACAACTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCA
TCGTTGAAAGCCGCACTGCACCAGACAGAAGCCTTAGCAGAGCAAGCCGCAGCCAGAGTGACAGCACTGG
TGGCTAAAAAAGCTCATTTGCAATATCTAAGGGACTTTAAATTGAATCCTAACCGCCTTCAAGTGATACG
TGAGCGCATTGATAATACTAAGCAAGATTTGGCTAAAACTACCTCATCTTTGTTAAATGCACAAGAAGCT
TTAGCAGCCTTACAAGCTAAACAAAGCAGTCTAGAAGCTACTATTGCTACCACAGAACACCAGTTGACTT
TGCTTAAAACCTTAGCTAACGAAAAGGAATATCGCCACTTAGACGAAGATATAGCTACTGTGCCTGATTT
GCAAGTAGCTCCACCTCTTACGGGCGTAAAACCGCTATCATATAGTAAGATAGATACTACTCCGCTTGTT
CAAGAAATGGTTAAAGAAACGAAACAACTATTAGAAGCTTCAGCAAGATTAGCTGCTGAAAATACAAGTC
TTGTAGCAGAAGCGCTTGTTGGCCAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGTCTAAAATCAC
ATCTTCGATTACTCAGCCCTCATCTAAGACATCTTATGGCTCAGGATCTTCTACAACGAGCAATCTCATT
TCTGATGTTGATGAAAGTACTCAAAGAGCTCTTAAAGCAGGAGTCGTCATGTTGGCAGCTGTCGGCCTCA
CAGGATTTAGGTTCCGTAAGGAATCTAAGTGA

Preferred GAS 040 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 9; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 9, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These GAS 040 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 9. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 9. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 9. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 9 is removed. As another example, in one embodiment, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 9 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

Further illustration of domains within GAS 40 is shown in FIGURES 1 and 2. As shown in these figures, GAS 40 contains a leader peptide sequence within amino acids 1 – 26, a coiled-coil region within amino acids 58 – 261, a coiled coil region within amino acids 556 – 733, a leucine zipper region within amino acids 673 – 701 and a transmembrane region within amino acids 855 – 866.

The coiled-coil regions of GAS 40 are likely to be involved in the formation of oligomers such as dimers or trimers. Such oligomers could be homomers (containing two or more GAS 40 proteins oligomerized together) or heteromers (containing one or more additional GAS proteins oligomerized with GAS 40).

Accordingly, in one embodiment, the combinations of the invention include a GAS 40 antigen in the form of an oligomer. The oligomer may comprise two more GAS 40 antigens or fragments thereof, or it may comprise GAS 40 or a fragment thereof oligomerized to a second GAS antigen. Preferably, a GAS 40 fragment used within an oligomer includes a portion of one of the coiled coil or leucine zipper domains.

*(6) GAS 389*

GAS 389 corresponds to M1 GenBank accession numbers GI:13622996 and GI:15675772, to M3 GenBank accession number GI: 21911237, to M18 GenBank accession number GI: 19746884, and is also referred to as 'Spy1981' (M1), 'SpyM3_1701' (M3), 'SpyM18_2045' (M18) and 'relA'. GAS 389 has also been identified as a (p)ppGpp synthetase. Amino acid and polynucleotide sequences of GAS 389 from an M1 strain are set forth below:

**SEQ ID NO: 11**

MRNEMAKIMNVTGEEVIALAATYMTKADVAFVAKALAYATAAHFYQVRKSGEPYIVHPIQVAGILADLHL
DAVTVACGFLHDVVEDTDITLDEIEADFGHDARDIVDGVTKLGEVEYKSHEEQLAENHRKMLMAMSKDIR
VILVKLADRLHNMRTLKHLRKDKQERISRETMEIYAPLAHRLGISRIKWELEDLAFRYLNETEFYKISHM
MKEKRRERELVEAIVSKVKTYTTQQGLFGDVYGRPKHIYSIYRKMRDKKKRFDQIFDLIAIRCVMETQS
DVYAMVGYIHELWRPMPGRFKDYIAAPKANGYQSIHTTVYGPKGPIEIQIRTKDMHQVAEYGVAAHWAYK

KGVRGKVNQAEQAVGMNWIKELVELQDASNGDAVDFVDSVKEDIFSERIYVFTPTGAVQELPKESGPIDF
AYAIHTQIGEKATGAKVNGRMVPLTAKLKTGDVVEIITNANSFGPSRDWVKLVKTNKARNKIRQFFKNQD
KELSVNKGRDLLVSYFQEQGYVANKYLDKKRIEAILPKVSVKSEESLYAAVGFGDISPISVFNKLTEKER
REEERAKAKAEAEELVKGGEVKHENKDVLKVRSENGVIIQGASGLLMRIAKCCNPVPGDPIDGYITKGRG
IAIHRSDCHNIKSQDGYQERLIEVEWDLDNSSKDYQAEIDIYGLNRSGLLNDVLQILSNSTKSISTVNAQ
PTKDMKFANIHVSFGIPNLTHLTTVVEKIKAVPDVYSVKRTNG

**SEQ ID NO: 12**

ATGAGGAACGAAATGGCAAAAATAATGAACGTAACAGGAGAAGAAGTCATTGCCTTAGCGGCCACCTATA
TGACCAAGGCTGATGTGGCTTTTGTGGCAAAGGCTTTAGCATATGCAACAGCGGCCCATTTCTACCAAGT
GAGAAAGTCAGGCGAACCCTATATCGTCCATCCGATTCAGGTGGCGGGGATTCTGGCTGATTTGCATCTG
GATGCTGTGACAGTTGCTTGTGGCTTTTTACATGATGTCGTAGAAGATACGGATATTACCTTAGATGAGA
TCGAAGCAGACTTTGGCCATGATGCTCGTGATATCGTTGATGGTGTCACCAAGTTAGGTGAAGTTGAGTA
CAAATCTCATGAGGAGCAACTCGCCGAAAACCATCGCAAAATGCTGATGGCTATGTCCAAAGATATTCGC
GTGATTTTGGTGAAATTGGCTGACCGCCTGCATAATATGCGCACCCTCAAACATTTGCGCAAGGACAAAC
AAGAGCGCATTTCGCGCGAAACCATGGAAATCTATGCCCCCTTGGCGCATCGTTTGGGGATTAGTCGCAT
CAAATGGGAACTAGAAGATTTGGCTTTTCGTTACCTCAATGAAACCGAATTTTACAAAATTTCCCATATG
ATGAAAGAAAAACGTCGCGAGCGTGAAGCTTTGGTAGAGGCTATTGTCAGTAAGGTCAAAACCTATACGA
CACAACAAGGGTTGTTTGGAGATGTGTATGGCCGACCAAAACACATTTATTCGATTTATCGGAAAATGCG
GGACAAAAAGAAACGATTCGATCAGATTTTTGATCTGATTGCCATTCGTTGTGTCATGGAAACGCAAAGC
GATGTCTATGCTATGGTTGGCTATATTCATGAGCTTTGGCGTCCCATGCCAGGCCGCTTCAAGGATTATA
TTGCAGCTCCTAAAGCTAATGGCTACCAGTCTATTCATACCACCGTGTATGGGCCAAAAGGACCTATTGA
GATTCAAATCAGAACTAAGGACATGCATCAAGTGGCTGAGTACGGGGTTGCTGCTCACTGGGCTTATAAA
AAAGGCGTGCGTGGTAAGGTCAATCAAGCTGAGCAAGCCGTTGGCATGAACTGGATCAAAGAGCTGGTAG
AATTGCAAGATGCCTCAAATGGCGATGCAGTGGACTTTGTGGATTCGGTCAAAGAAGACATTTTTTCTGA
ACGGATTTATGTCTTTACACCGACAGGGGCCGTTCAGGAGTTACCAAAAGAATCAGGTCCTATTGATTTT
GCTTATGCGATCCATACGCAAATCGGTGAAAAAGCAACAGGTGCCAAAGTCAATGGACGTATGGTTCCTC
TCACTGCCAAGTTAAAAACAGGAGATGTGGTTGAAATCATCACCAATGCCAATTCCTTTGGCCCTAGTCG
AGACTGGGTAAAACTGGTCAAAACCAATAAGGCTCGCAACAAAATTCGTCAGTTCTTTAAAAATCAAGAC
AAGGAATTGTCAGTGAATAAAGGCCGTGATTTGTTGGTGTCTTATTTTCAAGAGCAGGGCTACGTTGCCA
ATAAATACCTTGACAAAAAACGCATTGAAGCCATCCTTCCAAAAGTCAGTGTGAAGAGCGAAGAATCACT
CTATGCAGCCGTTGGGTTTGGTGACATTAGTCCTATCAGTGTCTTTAACAAGTTAACCGAAAAAGAGCGC
CGTGAAGAAGAAAGGGCCAAGGCTAAAGCAGAAGCTGAAGAATTGGTTAAGGGCGGTGAGGTCAAACACG
AAAACAAAGATGTGCTCAAGGTTCGCAGTGAAAATGGAGTCATTATCCAAGGAGCATCAGGCCTCTTGAT
GCGGATTGCCAAGTGTTGTAATCCTGTACCTGGTGATCCTATTGACGGCTACATTACCAAAGGGCGTGGC
ATTGCGATTCACAGATCGGACTGTCATAACATTAAGAGTCAAGATGGCTACCAAGAACGCTTGATTGAGG
TCGAGTGGGATTTGGACAATTCGAGTAAAGATTATCAGGCTGAAATTGATATCTATGGGCTCAATCGTAG
TGGTCTGCTTAATGATGTGCTCCAAATTTTATCAAACTCAACCAAGAGCATATCGACAGTCAATGCTCAG
CCGACCAAGGACATGAAGTTTGCTAATATTCACGTGAGCTTTGGCATTCCAAATCTGACGCATCTGACCA
CTGTTGTCGAAAAAATCAAGGCAGTTCCAGATGTTTATAGCGTGAAGCGGACCAATGGCTAA

Preferred GAS 389 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 11; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 11, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These GAS 389 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 11. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 11. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 11. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(7) GAS 504*

GAS 504 corresponds to M1 GenBank accession numbers GI:13622806 and GI:15675600, to M3 GenBank accession number GI: 21911061, to M18 GenBank accession number GI: 19746708, and is also referred to as 'Spy1751' (M1), 'SpyM3_1525', 'SpyM18_1823' (M18) and 'fabK'. GAS 504 has also been identified as a putative trans-2-enoyl-ACP reductase II. Amino acid and polynucleotide sequences of GAS 504 of an M1 strain are set forth below:

**SEQ ID NO: 13**

MKTRITELLNIDYPIFQGGMAWVADGDLAGAVSNAGGLGIIGGGNAPKEVVKANIDRVKAITDRPFGVNI
MLLSPFADDIVDLVIEEGVKVVTTGAGNPGKYMERLHQAGIIVVPVVPSVALAKRMEKLGVDAVIAEGME
AGGHIGKLTTMSLVRQVVEAVSIPVIAAGGIADGHGAAAAFMLGAEAVQIGTRFVVAKESNAHQNFKDKI
LAAKDIDTVISAQVVGHPVRSIKNKLTSAYAKAEKAFLIGQKTATDIEEMGAGSLRHAVIEGDVVNGSVM
AGQIAGLVRKEESCETILKDIYYGAARVIQNEAKRWQSVSIEK

**SEQ ID NO: 14**

ATGAAAACACGTATTACAGAATTACTTAATATTGATTACCCCATTTTTCAAGGAGGAATGGCTTGGGTTG
CTGATGGTGATTTAGCAGGTGCAGTTTCTAATGCTGGTGGTTTAGGCATTATAGGTGGTGGCAATGCTCC
CAAAGAAGTCGTTAAAGCTAATATTGATCGTGTCAAAGCTATTACTGATAGACCTTTTGGGGTTAATATC
ATGCTTTTATCTCCTTTTGCTGATGATATCGTTGATCTGGTCATTGAAGAAGGTGTTAAAGTAGTAACAA
CAGGCGCAGGAAATCCAGGAAAGTATATGGAAAGACTGCACCAGGCGGGTATAATCGTTGTTCCTGTTGT
CCCAAGCGTTGCGCTAGCCAAACGTATGGAAAAGCTTGGGGTAGATGCTGTTATTGCTGAGGGTATGGAA
GCTGGAGGACATATTGGCAAGTTAACGACTATGTCTTTAGTAAGACAAGTTGTTGAAGCGGTTTCGATTC
CTGTCATTGCGGCAGGTGGTATAGCTGATGGTCATGGTGCAGCAGCAGCATTTATGTTAGGAGCAGAGGC
TGTTCAAATTGGAACTCGCTTTGTTGTTGCTAAAGAATCCAATGCTCACCAAAATTTTAAAGATAAAATC
TTAGCAGCAAAAGATATTGATACGGTGATTTCTGCGCAGGTTGTGGGCCACCCTGTCCGTTCTATTAAAA
ATAAATTGACCTCAGCTTACGCTAAAGCAGAAAAAGCATTTTTAATTGGTCAAAAAACAGCTACTGATAT
TGAAGAAATGGGAGCAGGATCGCTTCGACACGCTGTTATTGAAGGCGATGTAGTCAATGGATCTGTTATG
GCTGGCCAAATTGCAGGGCTTGTGAGAAAAGAAGAAAGCTGTGAAACGATTTTAAAAGATATTTATTATG
GTGCAGCTCGTGTTATTCAAAATGAAGCTAAGCGCTGGCAATCTGTTTCAATAGAAAAGTAG

Preferred GAS 504 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 13, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 504 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 13. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 13. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 13. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(8) GAS 509*

GAS 509 corresponds to M1 GenBank accession numbers GI:13622692 and GI:15675496, to M3 GenBank accession number GI: 21910899, to M18 GenBank accession number GI: 19746544, and is also referred to as 'Spy1618' (M1), 'SpyM3_1363' (M3), 'SpyM18_1627' (M18) and 'cysM'. GAS 509 has also been identified as a putative O-acetylserine lyase. Amino acid and polynucleotide sequences of GAS 509 of an M1 strain are set forth below:

**SEQ ID NO: 15**

MTKIYKTITELVGQTPIIKLNRLIPNEAADVYVKLEAFNPGSSVKDRIALSMIEAAEAEGLISPGDVIIE
PTSGNTGIGLAWVGAAKGYRVIIVMPETMSLERRQIIQAYGAELVLTPGAEGMKGAIAKAETLAIELGAW
MPMQFNNPANPSIHEKTTAQEILEAFKEISLDAFVSGVGTGGTLSGVSHVLKKANPETVIYAVEAEESAV
LSGQEPGPHKIQGISAGFIPNTLDTKAYDQIIRVKSKDALETARLTGAKEGFLVGISSGAALYAAIEVAK
QLGKGKHVLTILPDNGERYLSTELYDVPVIKTK

**SEQ ID NO: 16**

ATGACTAAAATTTACAAAACTATAACAGAATTAGTAGGTCAAACACCTATTATCAAACTTAACCGTTTAA
TTCCAAACGAAGCTGCTGACGTTTATGTAAAATTAGAAGCTTTTAACCCAGGATCTTCTGTTAAAGATCG
TATTGCTTTATCGATGATTGAAGCTGCTGAAGCTGAAGGTCTGATAAGTCCTGGTGACGTTATTATCGAA
CCAACAAGTGGTAATACAGGTATTGGTCTTGCATGGGTAGGTGCTGCTAAAGGGTATCGAGTCATTATTG
TTATGCCCGAAACTATGAGCTTGGAAAGACGGCAAATCATTCAGGCTTATGGTGCAGAGCTTGTCTTAAC
ACCTGGAGCAGAAGGTATGAAAGGGGCTATTGCAAAAGCTGAAACTTTAGCAATAGAACTAGGTGCTTGG
ATGCCTATGCAATTTAATAACCCTGCCAATCCAAGCATCCATGAAAAAACAACAGCTCAAGAAATTTTGG
AAGCTTTTAAGGAGATTTCTTTAGATGCATTCGTATCTGGTGTTGGTACTGGAGGAACACTTTCTGGTGT
TTCACATGTCTTGAAAAAAGCTAACCCTGAAACTGTTATCTATGCTGTTGAAGCTGAAGAATCTGCTGTC
TTATCTGGTCAAGAGCCTGGACCACATAAAATTCAAGGTATATCAGCTGGATTTATCCCAAACACGTTAG
ATACCAAAGCCTATGACCAAATTATCCGTGTTAAATCGAAAGATGCTTTAGAAACTGCTCGACTAACAGG
AGCTAAGGAAGGCTTCCTGGTTGGGATTTCTTCTGGAGCTGCTCTTTACGCCGCTATTGAAGTCGCTAAA
CAGTTAGGAAAAGGCAAACATGTGTTAACTATTTTACCAGATAATGGCGAACGCTATTTATCGACTGAAC
TCTATGATGTACCAGTAATTAAGACGAAATAA

Preferred GAS 509 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 15; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 15, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 509 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 15. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 15. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 15. For example, in one embodiment, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 15 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(9) GAS 366***

GAS 366 corresponds to M1 GenBank accession numbers GI:13622612, GI:15675424 and GI:30315979, to M3 GenBank accession number GI: 21910712, to M18 GenBank accession number GI: 19746474, and is also referred to as 'Spy1525' (M1), 'SpyM3_1176' (M3), 'SpyM18_1542' (M18) and 'murD'. GAS 366 has also been identified as a UDP-N-acetylemuramoylalanine-D-glutamate ligase or a D-glutamic acid adding enzyme. Amino acid and polynucleotide sequences of GAS 366 of an M1 strain are set forth below:

**SEQ ID NO: 17**

MKVISNFQNKKILILGLAKSGEAAAKLLTKLGALVTVNDSKPFDQNPAAQALLEEGIKVICGSHPVELLD
ENFEYMVKNPGIPYDNPMVKRALAKEIPILTEVELAYFVSEAPIIGITGSNGKTTTTMIADVLNAGGQS
ALLSGNIGYPASKVVQKAIAGDTLVMELSSFQLVGVNAFRPHIAVITNLMPTHLDYHGSFEDYVAAKWMI
QAQMTESDYLILNANQEISATLAKTTKATVIPFSTQKVVDGAYLKDGILYFKEQAIIAATDLGVPGSHNI
ENALATIAVAKLSGIADDIIAQCLSHFGGVKHRLQRVGQIKDITFYNDSKSTNILATQKALSGFDNSRLI
LIAGGLDRGNEFDDLVPDLLGLKQMIILGESAERMKRAANKAEVSYLEARNVAEATELAPKLAQTGDTIL

LSPANASWDMYPNFEVRGDEFLATFDCLRGDA

**SEQ ID NO: 18**

ATGAAAGTGATAAGTAATTTTCAAAACAAAAAAATATTAATATTGGGGTTAGCCAAATCGGGCGAAGCAG
CAGCAAAATTATTGACCAAACTTGGTGCTTTAGTGACTGTTAATGATAGTAAACCATTTGACCAAAATCC
AGCGGCACAAGCCTTGTTGGAAGAGGGGATTAAGGTCATTTGTGGTAGCCACCCAGTAGAATTATTAGAT
GAGAACTTTGAGTACATGGTTAAAAACCCTGGGATTCCTTATGATAATCCTATGGTTAAACGCGCCCTTG
CAAAGGAAATTCCCATCTTGACTGAAGTAGAATTGGCTTATTTCGTATCTGAAGCGCCTATTATCGGGAT
TACAGGATCAAACGGGAAGACAACCACAACGACAATGATTGCCGATGTTTTGAATGCTGGCGGGCAATCT
GCACTCTTATCTGGAAACATTGGTTATCCTGCTTCAAAAGTTGTTCAAAAAGCAATTGCTGGTGATACTT
TGGTGATGGAATTGTCCTCTTTTCAATTAGTGGGAGTGAATGCTTTTCGCCCTCATATTGCTGTCATCAC
TAATTTAATGCCGACTCACCTGGACTATCATGGCAGTTTTGAGGATTATGTTGCTGCTAAATGGATGATT
CAAGCTCAGATGACAGAATCAGACTACCTTATTTTAAATGCTAATCAAGAGATTTCAGCAACTCTAGCTA
AGACCACCAAAGCAACAGTGATTCCTTTTTCAACTCAAAAAGTGGTTGATGGAGCTTATCTGAAGGATGG
AATACTCTATTTTAAAGAACAGGCGATTATAGCTGCAACTGACTTAGGTGTCCCAGGTAGCCACAACATT
GAAAATGCCCTAGCAACTATTGCAGTTGCCAAGTTATCTGGTATTGCTGATGATATTATTGCCCAGTGCC
TTTCACATTTTGGAGGCGTTAAACATCGTTTGCAACGGGTTGGTCAAATCAAAGATATTACCTTCTACAA
TGACAGTAAGTCAACCAATATTTTAGCCACTCAAAAAGCTTTATCAGGTTTTGATAACAGTCGCTTGATT
TTGATTGCTGGCGGTCTAGATCGTGGCAATGAATTTGACGATTTGGTGCCAGACCTTTTAGGACTTAAGC
AGATGATTATTTTGGGAGAATCCGCAGAGCGTATGAAGCGAGCTGCTAACAAAGCAGAGGTCTCTTATCT
TGAAGCTAGAAATGTGGCAGAAGCAACAGAGCTTGCTTTTAAGCTGGCCCAAACAGGCGATACTATCTTG
CTTAGCCCAGCCAATGCTAGCTGGGATATGTATCCTAATTTTGAGGTTCGTGGGGATGAATTTTTGGCAA
CCTTTGATTGTTTAAGAGGAGATGCCTAA

Preferred GAS 366 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 17; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 17, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 366 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 17. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 17. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 17. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 17 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(10) GAS 159***

GAS 159 corresponds to M1 GenBank accession numbers GI:13622244 and GI:15675088, to M3 GenBank accession number GI: 21910303, to M18 GenBank accession number GI: 19746056, and is also referred to as 'Spy1105' (M1), 'SpyM3_0767' (M3), 'SpyM18_1067' (M18) and 'potD'. GAS 159 has also been identified as a putative spermidine/putrescine ABC transporter (a periplasmic transport protein). Amino acid and polynucleotide sequences of GAS 159 of an M1 strain are set forth below:

**SEQ ID NO: 19**

MRKLYSFLAGVLGVIVILTSLSFILQKKSGSGSQSDKLVIYNWGDYIDPALLKKFTKETGIEVQYETFDS
NEAMYTKIKQGGTTYDIAVPSDYTIDKMIKENLLNKLDKSKLVGMDNIGKEFLGKSFDPQNDYSLPYFWG
TVGIVYNDQLVDKAPMHWEDLWRPEYKNSIMLIDGAREMLGVGLTTFGYSVNSKNLEQLQAAERKLQQLT
PNVKAIVADEMKGYMIQGDAAIGITFSGEASEMLDSNEHLHYIVPSEGSNLWFDNLVLPKTMKHEKEAYA
FLNFINRPENAAQNAAYIGYATPNKKAKALLPDEIKNDPAFYPTDDIIKKLEVYDNLGSRWLGIYNDLYL

QFKMYRK

**SEQ ID NO: 20**

ATGCGTAAACTTTATTCCTTTCTAGCAGGAGTTTTGGGTGTTATTGTTATTTTAACAAGTCTTTCTTTCA
TCTTGCAGAAAAAATCGGGTTCTGGTAGTCAATCGGATAAATTAGTTATTTATAACTGGGGAGATTACAT
TGATCCAGCTTTGCTCAAAAAATTCACCAAAGAAACGGGCATTGAAGTGCAGTATGAAACTTTCGATTCC
AATGAAGCCATGTACACTAAAATCAAGCAGGGCGGAACCACTTACGACATTGCTGTTCCTAGTGATTACA
CCATTGATAAAATGATCAAAGAAAACCTACTCAATAAGCTTGATAAGTCAAAATTAGTTGGCATGGATAA
TATCGGGAAAGAATTTTTAGGGAAAAGCTTTGACCCACAAAACGACTATTCTTTGCCTTATTTCTGGGGA
ACCGTTGGGATTGTTTATAATGATCAATTAGTTGATAAGGCGCCTATGCACTGGGAAGATCTGTGGCGTC
CAGAATATAAAAATAGTATTATGCTGATTGATGGAGCGCGTGAAATGCTAGGGGTTGGTTTAACAACTTT
TGGTTATAGTGTGAATTCTAAAAATCTAGAGCAGTTGCAGGCAGCCGAGAGAAAACTGCAGCAGTTGACG
CCGAATGTTAAAGCCATTGTAGCAGATGAGATGAAAGGCTACATGATTCAAGGTGACGCTGCTATTGGAA
TTACCTTTTCTGGTGAAGCCAGTGAGATGTTAGATAGTAACGAACACCTTCACTACATCGTGCCTTCAGA
AGGGTCTAACCTTTGGTTTGATAATTTGGTACTACCAAAAACCATGAAACACGAAAAAGAAGCTTATGCT
TTTTTGAACTTTATCAATCGTCCTGAAAATGCTGCGCAAAATGCTGCATATATTGGTTATGCGACACCAA
ATAAAAAAGCCAAGGCCTTACTTCCAGATGAGATAAAAAATGATCCTGCTTTTTATCCAACAGATGACAT
TATCAAAAAATTGGAAGTTTATGACAATTTAGGGTCAAGATGGTTGGGGATTTATAATGATTTATACCTC
CAATTTAAAATGTATCGCAAATAA

Preferred GAS 159 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 19; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 19, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 159 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 19. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 19. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 19. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 19 is removed. In another example, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 19 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(11) GAS 217***

GAS 217 corresponds to M1 GenBank accession numbers GI:13622089 and GI:15674945, to M3 GenBank accession number GI: 21910174, to M18 GenBank accession number GI: 19745987, and is also referred to as 'Spy0925' (M1), 'SpyM3_0638' (M3), and 'SpyM18_0982' (M18). GAS 217 has also been identified as a putative oxidoreductase. Amino acid and polynucleotide sequences of GAS 217 of an M1 strain are set forth below:

**SEQ ID NO: 21**

MAQRIIVITGASGGLAQAIVKQLPKEDSLILLGRNKERLEHCYQHIDNKECLELDITNPVAIEKMVAQIY
QRYGRIDVLINNAGYGAFKGFEEFSAQEIADMFQVNTLASIHFACLIGQKMAEQGQGHLINIVSMAGLIA
SAKSSIYSATKFALIGFSNALRLELADKGVYVTTVNPGPIATKFFDQADPSGHYLESVGKFTLQPNQVAK
RLVSIIGKNKRELNLPFSLAVTHQFYTLFPKLSDYLARKVFNYK

**SEQ ID NO: 22**

ATGGCACAAAGAATCATTGTTATCACGGGAGCTTCTGGAGGACTGGCTCAGGCAATTGTTAAGCAGTTAC
CCAAGGAAGACAGCTTGATTTTATTAGGACGTAACAAAGAACGCCTAGAACACTGTTATCAGCATATTGA

CAACAAAGAATGCCTCGAGTTGGATATTACCAATCCAGTAGCCATTGAGAAAATGGTCGCCCAGATTTAC
CAGCGCTATGGCCGTATTGATGTCTTGATTAATAATGCTGGCTACGGAGCTTTCAAAGGCTTTGAAGAGT
TTTCTGCCCAAGAAATAGCTGATATGTTTCAGGTTAACACCCTAGCGAGCATTCACTTTGCTTGCTTGAT
TGGTCAGAAAATGGCAGAGCAGGGGCAAGGTCACCTTATTAATATTGTGTCCATGGCAGGCTTGATTGCG
TCAGCCAAATCGAGCATTTATTCAGCCACCAAGTTTGCCCTTATCGGATTTTCCAATGCCCTTCGCTTAG
AATTAGCGGATAAAGGGGTTTACGTGACCACCGTGAATCCAGGTCCCATTGCCACCAAGTTTTTTGACCA
AGCTGACCCGTCTGGACATTATTTGGAAAGCGTTGGTAAATTTACTCTCCAACCAAATCAAGTGGCTAAG
CGTTTGGTTTCTATTATCGGGAAAAATAAACGAGAATTGAATTTGCCCTTTAGTTTAGCGGTGACCCATC
AATTTTACACCCTTTTCCCTAAATTATCTGATTATCTTGCAAGAAAGGTATTTAATTATAAATGA

Preferred GAS 217 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 21; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 21, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 217 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 21. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 21. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 21. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(12) GAS 309***

GAS 309 corresponds to M1 GenBank accession numbers GI:13621426 and GI:15674341, to M3 GenBank accession number GI: 21909633, to M18 GenBank accession number GI: 19745363, and is also referred to as 'Spy0124' (M1), 'SpyM3_0097' (M3), 'SpyM18_0205' (M18), 'nra' and 'rofA'. GAS 309 has also been identified as a regulatory protein and a negative transcriptional regulator. Amino acid and polynucleotide sequences of GAS 309 of an M1 strain are set forth below:

**SEQ ID NO: 23**

MIEKYLESSIESKCQLIVLFFKTSYLPITEVAEKTGLTFLQLNHYCEELNAFFPGSLSMTIQKRMISCQF
THPFKETYLYQLYASSNVLQLLAFLIKNGSHSRPLTDFARSHFLSNSSAYRMREALIPLLRNFELKLSKN
KIVGEEYRIRYLIALLYSKFGIKVYDLTQQDKNTIHSFLSHSSTHLKTSPWLSESFSFYDILLALSWKRH
QFSVTIPQTRIFQQLKKLFVYDSLKKSSHDIIETYCQLNFSAGDLDYLYLIYITANNSFASLQWTPEHIR
QYCQLFEENDTFRLLLNPIITLLPNLKEQKASLVKALMFFSKSFLFNLQHFIPETNLFVSPYYKGNQKLY
TSLKLIVEEWMAKLPGKRDLNHKHFHLFCHYVEQSLRNIQPPLVVVFVASNFINAHLLTDSFPRYFSDKS
IDFHSYYLLQDNVYQIPDLKPDLVITHSQLIPFVHHELTKGIAVAEISFDESILSIQELMYQVKEEKFQA
DLTKQLT

**SEQ ID NO: 24**

TTGATAGAAAAATACTTGGAATCATCAATCGAATCAAAATGTCAGTTAATTGTCTTGTTTTTTAAGACAT
CTTATTTGCCAATAACTGAGGTAGCAGAAAAAACTGGCTTAACCTTTTTACAACTAAACCATTATTGTGA
GGAACTGAATGCCTTTTTCCCTGGTAGTCTGTCTATGACCATCCAAAAAAGGATGATATCTTGCCAATTT
ACACATCCTTTTAAAGAAACTTATCTTTACCAACTCTATGCATCATCTAATGTCTTACAATTACTAGCCT
TTTTAATAAAAAATGGTTCCCACTCTCGTCCCCTTACGGATTTTGCAAGAAGTCATTTTTTATCAAACTC
CTCAGCTTATCGGATGCGCGAAGCATTGATTCCTTTATTAAGAAACTTTGAATTAAAACTCTCTAAGAAC
AAGATTGTCGGTGAGGAATATCGCATCCGTTACCTCATCGCTCTGCTATATAGTAAGTTTGGCATTAAAG
TTTATGACTTGACGCAGCAAGACAAAAACACTATTCATAGCTTTTTATCCCATAGTTCCACCCACCTTAA
AACCTCTCCTTGGTTATCGGAATCGTTTTCTTTCTATGACATTTTATTAGCTTTATCGTGGAAGCGGCAT
CAATTTTCGGTAACTATTCCCCAAACCAGAATTTTTCAACAATTAAAAAAACTTTTTGTCTACGATTCTT
TGAAAAAAAGTAGCCATGATATTATCGAAACTTACTGCCAACTAAACTTTTCAGCAGGAGATTTGGACTA
CCTCTATTTAATTTATATCACCGCTAATAATTCTTTTGCGAGCTTACAATGGACACCTGAGCATATCAGA

CAATATTGTCAACTTTTTGAAGAAAATGATACTTTTCGCCTGCTTTTAAATCCTATCATCACTCTTTTAC
CTAACCTAAAAGAGCAAAAGGCTAGTTTAGTAAAAGCTCTTATGTTTTTTTCAAAATCATTCTTGTTTAA
TCTGCAACATTTTATTCCTGAGACCAACTTATTCGTTTCTCCGTACTATAAAGGAAACCAAAAACTCTAT
ACGTCCTTAAAGTTAATTGTCGAAGAGTGGATGGCCAAACTTCCTGGTAAGCGTGACTTGAACCATAAGC
ATTTTCATCTTTTTTGCCACTATGTCGAGCAAAGTCTAAGAAATATCCAACCTCCTTTAGTTGTTGTTTT
CGTAGCCAGTAATTTTATCAATGCTCATCTCCTAACGGATTCTTTTCCAAGGTATTTCTCGGATAAAAGC
ATTGATTTTCATTCCTATTATCTATTGCAAGATAATGTTTATCAAATTCCTGATTTAAAGCCAGATTTGG
TCATCACTCACAGTCAACTGATTCCTTTTGTTCACCATGAACTTACAAAAGGAATTGCTGTTGCTGAAAT
ATCTTTTGATGAATCGATTCTGTCTATCCAAGAATTGATGTATCAAGTTAAAGAGGAAAAATTCCAAGCT
GATTTAACCAAGCAATTAACATAA

Preferred GAS 309 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 23; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 23, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 309 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 23. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 23. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 23. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(13) GAS 372*

GAS 372 corresponds to M1 GenBank accession numbers GI:13622698 and GI:15675501, to M3 GenBank accession number GI: 21910905, to M18 GenBank accession number GI: 19746500 and is also referred to as 'Spy1625' (M1), 'SpyM3_1369' (M3), and 'SpyM18_1634' (M18). GAS 372 has also been identified as a putative protein kinase or a putative eukaryotic-type serine/threonine kinase. Amino acid and polynucleotide sequences of GAS 372 of an M1 strain are set forth below:

**SEQ ID NO: 25**

MIQIGKLFAGRYRILKSIGRGGMADVYLANDLILDNEDVAIKVLRTNYQTDQVAVARFQREARAMAELNH
PNIVAIRDIGEEDGQQFLVMEYVDGADLKRYIQNHAPLSNNEVVRIMEEVLSAMTLAHQKGIVHRDLKPQ
NILLTKEGVVKVTDFGIAVAFAETSLTQTNSMLGSVHYLSPEQARGSKATIQSDIYAMGIMLFEMLTGHI
PYDGDSAVTIALQHFQKPLPSIIEENHNVPQALENVVIRATAKKLSDRYGSTFEMSRDLMTALSYNRSRE
RKIIFENVESTKPLPKVASGPTASVKLSPPTPTVLTQESRLDQTNQTDALQPPTKKKKSGRFLGTLFKIL
FSFFIVGVALFTYLILTKPTSVKVPNVAGTSLKVAKQELYDVGLKVGKIRQIESDTVAEGNVVRTDPKAG
TAKRQGSSITLYVSIGNKGFDMENYKGLDYQEAMNSLIETYGVPKSKIKIERIVTNEYPENTVISQSPSA
GDKFNPNGKSKITLSVAVSDTITMPMVTEYSYADAVNTLTALGIDASRIKAYVPSSSSATGFVPIHSPSS
KAIVSGQSPYYGTSLSLSDKGEISLYLYPEETHSSSSSSSTSSSNSSSINDSTAPGSNTELSPSETTSQ
TP

**SEQ ID NO: 26**

ATGATTCAGATTGGCAAATTATTTGCTGGTCGTTATCGCATTCTGAAATCTATTGGCCGCGGTGGTATGG
CGGATGTTTATTTAGCAAATGACTTGATCTTGGATAATGAAGACGTTGCAATCAAGGTCTTGCGTACCAA
TTATCAAACAGATCAGGTAGCAGTTGCCGCGTTTCCAACGAGAAGCGCGGGCCATGGCTGAATTGAACCAT
CCCAATATTGTTGCCATCCGGGATATAGGTGAAGAAGACGGACAGCAATTTTTAGTAATGGAATATGTGG
ATGGTGCTGACCTAAAGAGATACATTCAAAATCATGCTCCATTATCTAATAATGAAGTGGTTAGAATTAT
GGAAGAAGTCCTTTCTGCTATGACTTTAGCCCACCAAAAAGGAATTGTACACAGAGATTTAAAACCTCAA
AATATCCTACTAACTAAGGAGGGTGTTGTCAAAGTAACTGATTTCGGCATCGCAGTAGCCTTTGCAGAAA
CAAGCTTGACACAAACTAATTCGATGTTAGGCAGTGTTCATTACTTGTCTCCAGAACAGGCTCGCGGCTC
CAAAGCGACGATTCAAAGTGATATTTATGCGATGGGGATTATGCTCTTTGAGATGTTGACAGGCCATATC

CCTTATGACGGCGATAGTGCTGTTACGATTGCCTTGCAACATTTTCAAAAGCCTCTTCCATCTATTATCG
AGGAGAACCACAATGTGCCACAAGCTTTGGAGAATGTTGTTATTCGAGCAACAGCCAAGAAATTAAGTGA
TCGTTACGGGTCAACCTTTGAAATGAGTCGTGACTTAATGACGGCGCTTAGTTATAATCGTAGTCGGGAG
CGTAAGATTATCTTTGAGAATGTTGAAAGTACCAAACCCCTCCCCAAAGTGGCCTCAGGTCCCACCGCTT
CTGTAAAATTGTCTCCCCCTACCCCAACAGTGTTAACACAGGAAAGTCGATTAGATCAAACTAATCAAAC
AGATGCTTTACAGCCCCCCACCAAAAAGAAAAAAAGTGGTCGTTTTTTAGGTACTTTATTCAAAATTCTT
TTTTCTTTCTTTATTGTAGGTGTAGCACTCTTTACTTATCTTATACTAACTAAACCAACTTCTGTGAAAG
TTCCTAATGTAGCAGGCACTAGTCTTAAAGTTGCCAAACAAGAACTGTATGATGTTGGGCTAAAAGTGGG
TAAAATCAGGCAAATTGAGAGTGATACGGTTGCTGAGGGAAATGTAGTTAGAACAGATCCTAAAGCAGGA
ACAGCTAAGAGGCAAGGCTCAAGCATTACGCTTTATGTGTCAATTGGAAACAAAGGTTTTGACATGGAAA
ACTACAAAGGACTAGATTATCAAGAAGCTATGAATAGTTTGATAGAAACTTATGGTGTTCCAAAATCAAA
AATCAAAATTGAGCGCATTGTAACTAATGAATATCCTGAAAATACAGTCATCAGTCAATCGCCAAGTGCG
GGTGATAAATTTAATCCAAACGGAAAGTCTAAAATTACGCTCAGTGTTGCTGTTAGTGATACGATCACTA
TGCCTATGGTAACAGAATATAGTTATGCAGATGCAGTCAATACCTTAACAGCTTTAGGTATAGATGCATC
TAGAATAAAAGCTTATGTGCCAAGCTCTAGCTCAGCAACGGGCTTTGTGCCAATTCATTCTCCTAGTTCT
AAAGCTATTGTCAGTGGTCAATCTCCTTACTATGGAACGTCTTTGAGTCTGTCTGATAAAGGAGAGATTA
GTCTTTACCTTTATCCAGAAGAAACACACTCTTCTAGTAGCTCATCGAGTTCAACGTCAAGTTCAAACAG
TTCTTCAATAAATGATAGTACTGCACCAGGTAGCAACACTGAATTAAGCCCATCAGAAACTACTTCTCAA
ACACCTTAA

Preferred GAS 372 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 25; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 25, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These GAS 372 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 25. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 25. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 25. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(14) GAS 039*

GAS 039 corresponds to M1 GenBank accession numbers GI:13621542 and GI:15674446, to M3 GenBank accession number GI: 21909730, to M18 GenBank accession number GI: 19745398 and is also referred to as 'Spy0266' (M1), 'SpyM3_0194' (M3), and 'SpyM18_0250' (M18). Amino acid and polynucleotide sequences of GAS 039 of an M1 strain are set forth below:

**SEQ ID NO: 27**

MDLILFLLVLVLLGLGAYLLFKVNGLQHQLAQTLEGNADNLSDQMTYQLDTANKQQLLELTQLMNRQQAG
LYQQLTDIRDVLHRSLSDSRDRSDKRLEKINQQVNQSLKNMQESNEKRLEKMRQIVEEKLEETLKNRLHA
SFDSVSKQLESVNKGLGEMRSVAQDVGTLNKVLSNTKTRGILGELQLGQIIEDIMTSSQYEREFVTVSGS
SERVEYAIKLPGNGQGGYIYLPIDSKFPLEDYYRLEDAYEVGDKLAIEASRKALLAAIKRFAKDIHKKYL
NPPETTNFGVMFLPTEGLYSEVVRNASFFDSLRREENIVVAGPSTLSALLNSLSVGFKTLNIQKNADDIS
KILGNVKLEFDKFGGLLAKAQKQMNTANNTLDQLISTRTNAIVRALNTVETYQDQATKSLLNMPLLEEEN
NEN

**SEQ ID NO: 28**

ATGGACCTTATCTTGTTCCTTTTGGTCTTGGTTCTCTTAGGTTTAGGGGCTTATCTGTTGTTCAAAGTCA
ACGGCCTTCAACATCAGCTTGCCCAAACCCTAGAAGGCAACGCGGATAATTTGTCTGACCAAATGACCTA
CCAGTTGGATACAGCTAACAAACAACAATTGTTAGAGCTAACACAGCTGATGAACCGACAACAAGCAGGC
CTTTACCAACAATTAACAGATATTCGTGACGTCTTGCACCGTAGTTTGTCTGATAGTAGGGACCGGTCTG

ACAAACGCTTAGAAAAAATTAACCAGCAGGTCAACCAATCGCTCAAAAATATGCAAGAATCTAACGAAAA
ACGTTTGGAGAAAATGCGCCAGATCGTTGAAGAAAAATTGGAAGAAACCTTAAAAAATCGTCTGCACGCC
TCTTTCGATTCTGTATCCAAGCAACTAGAAAGTGTCAATAAAGGCTTGGGAGAAATGCGTAGCGTGGCTC
AAGATGTGGGTACTTTAAATAAGGTTTTGTCCAATACCAAAACACGAGGCATTTTAGGCGAACTTCAACT
AGGCCAAATCATTGAGGATATCATGACATCAAGCCAGTACGAAAGAGAATTTGTAACGGTTAGTGGTTCT
AGTGAACGCGTAGAATATGCGATTAAGCTCCCAGGAAATGGTCAAGGCGGTTATATTTACCTACCGATTG
ACTCAAAATTCCCTCTTGAAGATTATTACCGATTAGAAGATGCTTACGAAGTTGGTGATAAACTGGCCAT
CGAGGCTAGCCGAAAAGCACTTCTGGCAGCTATCAAACGCTTTGCCAAAGACATTCATAAAAAGTACTTG
AACCCCCCAGAGACGACCAATTTCGGAGTTATGTTCTTACCAACAGAAGGTCTTTATTCAGAAGTGGTCA
GAAATGCGTCTTTCTTTGATAGCCTTCGTCGGGAAGAAAATATTGTGGTTGCAGGCCCTTCGACCCTGTC
TGCTTTGCTGAATTCCTTATCTGTTGGTTTCAAGACCCTTAATATCCAAAAAAATGCTGATGACATCAGT
AAAATTTTAGGCAATGTCAAGTTAGAATTCGATAAATTTGGCGGCCTGCTTGCCAAGGCTCAAAAACAAA
TGAATACAGCTAATAATACGCTGGATCAGCTCATTTCAACAAGGACAAATGCCATTGTTCGAGCCTTGAA
TACCGTTGAAACTTATCAAGACCAAGCAACAAAATCTCTCTTGAACATGCCCTTATTAGAAGAGGAAAAT
AATGAAAATTAA

Preferred GAS 039 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 27; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 27, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 039 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 27. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 27. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 27. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(15) GAS 042***

GAS 042 corresponds to M1 GenBank accession numbers GI:13621559 and GI:15674461, to M3 GenBank accession number GI: 21909745, to M18 GenBank accession number GI: 19745415, and is also referred to as 'Spy0287' (M1), 'SpyM3_0209' (M3), and 'SpyM18_0275' (M18). Amino acid and polynucleotide sequences of GAS 042 of an M1 strain are set forth below:

**SEQ ID NO: 29**

MTKEKLVAFSQAHAEPAWLQERRLAALEAIPNLELPTIERVKFHRWNLGDGTLTENESLASVPDFIAIGD
NPKLVQVGTQTVLEQLPMALIDKGVVFSDFYTALEEIPEVIEAHFGQALAFDEDKLAAYHTAYFNSAAVL
YVPDHLEITTPIEAIFLQDSDSDVPFNKHVLVIAGKESKFTYLERFESIGNATQKISANISVEVIAQAGS
QIKFSAIDRLGPSVTTYISRRGRLEKDANIDWALAVMNEGNVIADFDSDLIGQGSQADLKVVAASSGRQV
QGIDTRVTNYGQRTVGHILQHGVILERGTLTFNGIGHILKDAKGADAQQESRVLMLSDQARADANPILLI
DENEVTAGHAASIGQVDPEDMYYLMSRGLDQETAERLVIRGFLGAVIAEIPIPSVRQEIIKVLDEKLLNR

**SEQ ID NO: 30**

ATGACAAAAGAAAAACTAGTGGCTTTTTCGCAAGCCCACGCTGAGCCTGCTTGGCTGCAAGAACGGCGTT
TAGCGGCATTAGAAGCCATTCCAAATTTGGAATTACCAACCATCGAAAGGGTTAAATTTCACCGTTGGAA
TCTAGGAGATGGTACCTTAACAGAAAATGAAAGTCTAGCTAGTGTTCCAGATTTTATAGCTATTGGAGAT
AACCCAAAGCTTGTTCAGGTAGGCACGCAAACAGTCTTAGAACAGTTACCAATGGCGTTAATTGACAAGG
GAGTTGTTTTCAGTGATTTTTATACGGCGCTTGAGGAAATCCCAGAAGTAATTGAAGCTCATTTTGGTCA
GGCATTAGCTTTTGATGAAGACAAACTAGCTGCCTACCACACTGCTTATTTTAATAGCGCAGCCGTGCTC
TACGTTCCTGATCACTTGGAAATCACAACTCCTATTGAAGCTATTTTCTTACAAGATAGTGACAGTGACG
TTCCTTTTAACAAGCATGTTCTAGTGATTGCAGGAAAAGAAAGTAAGTTCACCTATTTAGAGCGTTTTGA
ATCTATTGGCAATGCCACTCAAAAGATCAGCGCTAATATCAGTGTAGAAGTGATTGCTCAAGCAGGCAGC
CAGATTAAATTCTCGGCTATCGACCGCTTAGGTCCTTCAGTGACAACCTATATTAGCCGTCGAGGACGTT

TAGAGAAGGATGCCAACATTGATTGGGCCTTAGCTGTGATGAATGAAGGCAATGTCATTGCTGATTTTGA
CAGTGATTTGATTGGTCAGGGCTCACAAGCTGATTTGAAAGTTGTTGCAGCCTCAAGTGGTCGTCAGGTA
CAAGGTATTGACACGCGCGTGACCAACTATGGTCAACGTACGGTCGGTCATATTTTACAGCATGGTGTGA
TTTTGGAACGTGGCACCTTAACGTTTAACGGGATTGGTCATATTCTAAAAGACGCTAAGGGAGCTGATGC
TCAACAAGAAAGCCGTGTTTTGATGCTTTCTGACCAAGCAAGAGCCGATGCCAATCCAATCCTCTTAATT
GATGAAAATGAAGTAACAGCAGGTCATGCAGCTTCTATCGGTCAGGTTGACCCTGAAGATATGTATTACT
TGATGAGTCGAGGACTGGATCAAGAAACAGCAGAACGATTGGTTATTAGAGGATTCCTAGGAGCGGTTAT
CGCTGAAATTCCTATTCCATCAGTCCGCCAAGAGATTATTAAGGTTTTAGATGAGAAATTGCTTAATCGT
TAA

Preferred GAS 042 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 29; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 29, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 042 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 29. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 29. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 29. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(16) GAS 058***

GAS 058 corresponds to M1 GenBank accession numbers GI:13621663 and GI:15674556, to M3 GenBank accession number GI: 21909841, to M18 GenBank accession number GI: 19745567 and is also referred to as 'Spy0430' (M1), 'SpyM3_0305' (M3), and 'SpyM18_0477' (M18). Amino acid and polynucleotide sequences of GAS 058 of an M1 strain are set forth below:

**SEQ ID NO: 31**
MKWSGFMKTKSKRFLNLATLCLALLGTTLLMAHPVQAEVISKRDYMTRFGLGDLEDDSANYPSNLEARYK
GYLEGYEKGLKGDDIPERPKIQVPEDVQPSDHGDYRDGYEEGFGEGQHKRDPLETEAEDDSQGGRQEGRQ
GHQEGADSSDLNVEESDGLSVIDEVVGVIYQAFSTIWTYLSGLF

**SEQ ID NO: 32**
ATGAAATGGAGTGGTTTTATGAAAACAAAATCAAAACGCTTTTTAAACCTAGCAACCCTTTGCTTGGCCC
TACTAGGAACAACTTTGCTAATGGCACATCCCGTACAGGCGGAGGTGATATCAAAAAGAGACTATATGAC
TCGCTTCGGGTTAGGCGATTTAGAAGATGATTCAGCTAACTATCCTTCAAATTTAGAAGCTAGATATAAA
GGATATTTAGAGGGATATGAAAAAGGCTTAAAAGGAGATGATATACCCGAACGGCCCAAGATTCAGGTTC
CTGAGGATGTTCAGCCATCTGACCATGGCGACTATAGAGATGGTTATGAGGAAGGATTTGGAGAAGGACA
ACATAAACGTGATCCATTAGAAACAGAAGCAGAAGATGATTCTCAAGGAGGACGTCAAGAAGGACGTCAA
GGACATCAAGAAGGAGCAGATTCTAGTGATTTGAACGTTGAAGAAAGCGACGGTTTGTCTGTTATTGATG
AAGTAGTTGGAGTAATTTATCAAGCATTTAGTACTATTTGGACATACTTAAGCGGTTTGTTCTAA

Preferred GAS 058 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 31; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 31, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 058 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 31. Preferred fragments

of (b) comprise an epitope from SEQ ID NO: 31. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 31. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 31 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(17) GAS 290*

GAS 290 corresponds to M1 GenBank accession numbers GI:13622978 and GI:15675757, to M3 GenBank accession number GI: 21911221, to M18 GenBank accession number GI: 19746869 and is also referred to as 'Spy1959' (M1), 'SpyM3_1685' (M3), and 'SpyM18_2026' (M18). Amino acid and polynucleotide sequences of GAS 290 of an M1 strain are set forth below:

**SEQ ID NO: 33**

MKHILFIVGSLREGSFNHQLAAQAQKALEHQAVVSYLNWKDVPVLNQDIEANAPLPVVDARQAVQSADAI
WIFTPVYNFSIPGSVKNLLDWLSRALDLSDPTGPSAIGGKVVTVSSVANGGHDQVFDQFKALLPFIRTSV
AGEFTKATVNPDAWGTGRLEISKETKANLLSQAEALLAAI

**SEQ ID NO: 34**

ATGAAACATATTTTATTTATTGTTGGCTCGCTTCGTGAAGGGTCTTTTAACCATCAATTAGCGGCTCAAG
CACAAAAAGCTCTGGAACATCAAGCAGTTGTATCTTACTTAAATTGGAAAGACGTTCCTGTTTTGAATCA
AGATATCGAAGCTAATGCACCTTTACCAGTTGTTGACGCTCGTCAAGCTGTTCAGTCAGCGGATGCTATC
TGGATTTTTACACCAGTTTACAACTTCTCTATTCCAGGTTCTGTTAAAAACCTGCTAGACTGGTTGTCTC
GTGCTCTTGATTTGTCTGATCCGACGGGCCCATCTGCTATTGGCGGTAAGGTGGTTACGGTCTCTTCAGT
TGCAAATGGCGGGCATGATCAAGTATTTGATCAGTTTAAAGCACTATTGCCGTTTATCCGAACTTCAGTA
GCAGGAGAGTTTACAAAAGCAACTGTGAATCCTGATGCCTGGGGAACAGGAAGGCTTGAGATTTCAAAAG
AGACAAAAGCAAACTTGCTATCTCAGGCAGAGGCTCTTTTAGCGGCTATTTAG

Preferred GAS 290 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 33; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 33, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 290 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 33. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 33. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 33. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(18) GAS 511*

GAS 511 corresponds to M1 GenBank accession numbers GI:13622798 and GI:15675592, to M3 GenBank accession number GI: 21911053, to M18 GenBank accession number GI: 19746700 and is also referred to as 'Spy1743' (M1), 'SpyM3_1517' (M3), 'SpyM18_1815' (M18) and 'accA'. Amino acid and polynucleotide sequences of GAS 511 of an M1 strain are set forth below:

**SEQ ID NO: 35**

MTDVSRILKEARDQGRLTTLDYANLIFDDFMELHGDRHFSDDGAIVGGLAYLAGQPVTVIGIQKGKNLQD
NLARNFGQPNPEGYRKALRLMKQAEKFGRPVVTFINTAGAYPGVGAEERGQGEAIAKNLMEMSDLKVPII
AIIIGEGGSGGALALAVADQVWMLENTMYAVLSPEGFASILWKDGSRATEAAELMKITAGELYKMGIVDR
IIPEHGYFSSEIVDIIKANLIEQITSLQAKPLDQLLDERYQRFRKY

**SEQ ID NO: 36**

ATGACAGATGTATCAAGAATTTTAAAAGAAGCGCGTGATCAAGGGCGTTTAACAACTTTGGATTACGCCA
ACCTTATTTTCGATGACTTTATGGAACTGCATGGCGATCGCCATTTTTCAGATGATGGTGCCATTGTAGG
TGGCCTAGCTTATTTGGCGGGACAACCTGTTACGGTCATTGGTATTCAAAAAGGTAAGAATTTACAGGAT
AATTTGGCAAGGAATTTTGGCCAGCCCAATCCAGAAGGTTATCGTAAAGCTTTGCGCCTTATGAAACAGG
CAGAAAAATTTGGACGACCAGTTGTTACGTTTATCAATACTGCAGGAGCCTATCCAGGTGTCGGTGCGGA
AGAACGAGGACAGGGTGAGGCCATTGCTAAAAATTTGATGGAAATGAGTGATCTCAAGGTTCCCATTATC
GCCATCATTATTGGTGAAGGAGGCTCTGGTGGTGCATTAGCCTTAGCGGTTGCCGATCAGGTCTGGATGC
TTGAAAATACTATGTATGCGGTTCTTAGCCCAGAAGGCTTTGCTTCTATTTTATGGAAGGATGGTTCAAG
GGCGACCGAGGCCGCTGAATTGATGAAAATCACAGCGGGTGAACTCTACAAAATGGGAATAGTAGACCGT
ATTATTCCAGAACATGGTTATTTTTCAAGTGAAATCGTTGACATCATCAAAGCTAACCTCATCGAACAAA
TAACCAGTTTGCAAGCTAAGCCATTAGACCAATTATTAGATGAGCGCTACCAACGCTTTCGTAAATATTA
A

Preferred GAS 511 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 35; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 35, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 511 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 35. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 35. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 35. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(19) GAS 533***

GAS 533 corresponds to M1 GenBank accession numbers GI:13622912 and GI:15675696, to M3 GenBank accession number GI: 21911157, to M18 GenBank accession number GI: 19746804 and is also referred to as 'Spy1877' (M1), 'SpyM3_1621' (M3), 'SpyM18_1942' (M18) and 'glnA'. GAS 533 has also been identified as a putative glutamine synthetase. Amino acid and polynucleotide sequences of GAS 533 of an M1 strain are set forth below:

**SEQ ID NO: 37**

MAITVADIRREVKEKNVTFLRLMFTDIMGVMKNVEIPATKEQLDKVLSNKVMFDGSSIEGFVRINESDMY
LYPDLDTWIVFPWGDENGAVAGLICDIYTAEGKPFAGDPRGNLKRALKHMNEIGYKSFNLGPEPEFFLFK
MDDKGNPTLEVNDNGGYFDLAPIDLADNTRREIVNILTKMGFEVEASHHEVAVGQHEIDFKYADVLKACD
NIQIFKLVVKTIAREHGLYATFMAKPKFGIAGSGMHCNMSLFDNQGNNAFYDEADKRGMQLSEDAYYFLG
GLMKHAYNYTAITNPTVNSYKRLVPGYEAPVYVAWAGSNRSPLIRVPASRGMGTRLELRSVDPTANPYLA
LAVLLEAGLDGIINKIEAPEPVEANIYTMTMEERNEAGIIDLPSTLHNALKALQKDDVVQKALGYHIYTN
FLEAKRIEWSSYATFVSQWEIDHYIHNY

**SEQ ID NO: 38**

ATGGCAATAACAGTAGCTGACATTCGTCGTGAAGTCAAAGAAAAAAATGTAACGTTTCTTCGCTTGATGT
TCACTGATATCATGGGCGTTATGAAAAATGTGGAGATTCCTGCAACTAAAGAACAGTTAGACAAAGTATT
GTCTAACAAGGTTATGTTTGATGGTTCATCTATCGAAGGTTTTGTACGGATCAATGAGTCAGATATGTAC

CTTTACCCCGATTTAGACACTTGGATTGTTTTTCCCTGGGGAGATGAAAATGGAGCAGTTGCAGGTTTAA
TTTGTGATATTTATACAGCAGAAGGAAAGCCTTTTGCAGGAGATCCTAGAGGAAATTTAAAAAGAGCCCT
GAAACACATGAACGAGATCGGCTACAAATCATTTAATCTTGGACCAGAACCAGAATTTTTCCTTTTTAAG
ATGGATGATAAAGGTAATCCGACACTTGAAGTTAACGATAATGGTGGTTATTTTGATTTAGCGCCAATTG
ACTTAGCAGACAACACGCGCCGTGAAATTGTGAATATTTTAACGAAAATGGGTTTTGAAGTGGAAGCTAG
TCATCATGAAGTGGCTGTTGGTCAACATGAGATTGATTTTAAATATGCAGATGTTTTGAAAGCTTGTGAT
AATATTCAAATTTTTAAGCTAGTTGTAAAAACGATTGCCCGTGAACATGGACTTTATGCTACTTTCATGG
CTAAACCAAAATTTGGAATAGCTGGATCAGGGATGCACTGTAACATGTCTTTGTTTGATAACCAAGGTAA
TAATGCTTTTTATGATGAAGCTGATAAGCGAGGGATGCAGTTATCAGAAGATGCTTATTATTTCTTGGGA
GGACTAATGAAGCATGCTTATAACTACACTGCTATCACTAACCCTACAGTGAATTCTTATAAACGATTAG
TTCCAGGTTATGAGGCACCTGTTTATGTCGCTTGGGCTGGAAGTAATCGTTCACCGCTTATCCGTGTTCC
AGCATCACGTGGTATGGGAACGCGTTTGGAGTTACGTTCGGTTGATCCGACAGCTAATCCTTATTTAGCC
TTGGCTGTTCTCTTGGAAGCTGGATTAGATGGTATCATTAACAAAATTGAAGCTCCAGAACCCGTTGAAG
CTAACATTTATACCATGACAATGGAAGAACGAAATGAAGCAGGCATTATTGATTTGCCATCAACGCTTCA
TAATGCCTTAAAAGCTCTTCAAAAAGATGATGTGGTACAAAAGGCACTAGGTTACCATATCTACACTAAT
TTCTTAGAAGCAAAACGAATTGAATGGTCTTCCTATGCAACTTTTGTTTCTCAATGGGAAATTGACCATT
ATATTCATAATTATTAG

Preferred GAS 533 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 37; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 37, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 533 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 37. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 37. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 37. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(20) GAS 527*

GAS 527 corresponds to M1 GenBank accession numbers GI:13622332, GI:15675169, and GI:24211764, to M3 GenBank accession number GI: 21910381, to M18 GenBank accession number GI: 19746136, and is also referred to as 'Spy1204' (M1), 'SpyM3_0845' (M3), 'SpyM18_1155' (M18) and 'guaA'. GAS 527 has also been identified as a putative GMP synthetase (glutamate hydrolyzing) (glutamate amidotransferase). Amino acid and polynucleotide sequences of GAS 527 of an M1 strain are set forth below:

**SEQ ID NO: 39**

MTEISILNDVQKIIVLDYGSQYNQLIARRIREFGVFSELKSHKITAQELREINPIGIVLSGGPNSVYADN
AFGIDPEIFELGIPILGICYGMQLITHKLGGKVVPAGQAGNREYGQSTLHLRETSKLFSGTPQEQLVLMS
HGDAVTEIPEGFHLVGDSNDCPYAAIENTEKNLYGIQFHPEVRHSVYGNDILKNFAISICGARGDWSMDN
FIDMEIAKIRETVGDRKVLLGLSGGVDSSVVGVLLQKAIGDQLTCIFVDHGLLRKDEGDQVMGMLGGKFG
LNIIRVDASKRFLDLLADVEDPEKKRKIIGNEFVYVFDDEASKLKGVDFLAQGTLYTDIIESGTETAQTI
KSHHNVGGLPEDMQFELIEPLNTLFKDEVRALGIALGMPEEIVWRQPFPGPGLAIRVMGAITEEKLETVR
ESDAILREEIAKAGLDRDVWQYFTVNTGVRSVGVMGDGRTYDYTIAIRAITSIDGMTADFAQLPWDVLKK
ISTRIVNEVDHVNRIVYDITSKPPATVEWE

**SEQ ID NO: 40**

ATGACTGAAATTTCAATTTTGAATGATGTTCAAAAAATTATCGTTCTTGATTATGGTAGCCAGTACAATC
AGCTTATTGCTAGACGTATTCGAGAGTTTGGTGTTTTCTCCGAACTAAAAAGCCATAAAATCACCGCTCA

AGAACTTCGTGAGATCAATCCCATAGGTATCGTTTTATCAGGAGGGCCTAACTCTGTTTACGCTGATAAC
GCCTTTGGCATTGACCCTGAAATCTTTGAACTAGGGATTCCGATTCTTGGTATCTGTTACGGTATGCAAT
TAATCACCCATAAATTAGGTGGTAAAGTTGTTCCTGCTGGACAAGCTGGTAATCGTGAATACGGTCAGTC
AACCCTTCATCTTCGTGAAACGTCAAAATTATTTTCAGGCACACCTCAAGAACAACTCGTTTTGATGAGC
CATGGTGATGCTGTTACTGAAATTCCAGAAGGTTTCCACCTTGTTGGAGACTCAAATGACTGTCCCTATG
CAGCTATTGAAAATACTGAGAAAAACCTTTACGGTATTCAGTTCCACCCAGAAGTGAGACACTCTGTTTA
TGGAAATGACATTCTTAAAAACTTTGCTATATCAATTTGTGGCGCGCGTGGTGATTGGTCAATGGATAAT
TTTATTGACATGGAAATTGCTAAAATTCGTGAAACTGTAGGCGATCGTAAAGTTCTTCTAGGTCTTTCTG
GTGGAGTTGATTCTTCAGTTGTTGGTGTTCTACTTCAAAAAGCTATCGGTGACCAATTAACTTGTATTTT
CGTTGATCACGGTCTTCTTCGTAAAGACGAGGGCGATCAAGTTATGGGAATGCTTGGGGGCAAATTTGGC
CTAAATATTATCCGTGTGGATGCTTCAAAACGTTTCTTAGACCTTCTTGCAGACGTTGAAGATCCTGAGA
AAAAACGTAAAATTATTGGTAATGAATTTGTCTATGTTTTTGATGATGAAGCCAGCAAATTAAAAGGTGT
TGACTTCCTTGCCCAAGGAACACTTTATACTGATATCATTGAGTCAGGAACAGAAACTGCTCAAACCATC
AAATCACATCACAATGTGGGTGGTCTCCCCGAAGACATGCAGTTTGAATTGATTGAGCCCTTAAACACTC
TTTTCAAAGATGAAGTTCGAGCGCTTGGAATCGCTCTTGGAATGCCTGAAGAAATTGTTTGGCGCCAACC
ATTTCCAGGTCCTGGACTTGCTATCCGTGTCATGGGAGCAATTACTGAAGAAAAACTTGAAACCGTTCGC
GAATCAGACGCTATCCTTCGTGAAGAAATTGCTAAGGCTGGACTTGATCGTGACGTGTGGCAATACTTTA
CAGTTAACACAGGTGTCCGTTCTGTAGGCGTCATGGGAGATGGTCGTACTTATGATTATACCATCGCCAT
TCGTGCTATTACGTCTATTGATGGTATGACAGCTGACTTTGCTCAACTTCGTTGGGATGTCTTGAAAAAA
ATCTCAACACGTATCGTAAATGAAGTTGACCACGTTAACCGTATCGTCTACGACATCACAAGTAAACCAC
CCGCAACAGTTGAATGGGAATAA

Preferred GAS 527 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 39; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 39, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 527 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 39. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 39. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 39. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(21) GAS 294*

GAS 294 corresponds to M1 GenBank accession numbers GI:13622306, GI:15675145, and GI:26006773, to M3 GenBank accession number GI: 21910357, to M18 GenBank accession number GI: 19746111 and is also referred to as 'Spy1173' (M1), 'SpyM3_0821' (M3), 'SpyM18_1125' (M18) and 'gid'. GAS 294 has also been identified as a putative glucose-inhibited division protein. Amino acid and polynucleotide sequences of GAS 294 of an M1 strain are set forth below:

**SEQ ID NO: 41**

MSQSTATYINVIGAGLAGSEAAYQIAKRGIPVKLYEMRGVKATPQHKTTNFAELVCSNSFRGDSLTNAVG
LLKEEMRRLDSIIMRNGEANRVPAGGAMAVDREGYAESVTAELENHPLIEVIRGEITEIPDDAITVIATG
PLTSDALAEKIHALNGGDGFYFYDAAAPIIDKSTIDMSKVYLKSRYDKGEAAYLNCPMTKEEFMAFHEAL
TTAEEAPLNAFEKEKYFEGCMPIEVMAKRGIKTMLYGPMKPVGLEYPDDYTGPRDGEFKTPYAVVQLRQD
NAAGSLYNIVGFQTHLKWGEQKRVFQMIPGLENAEFVRYGVMHRNSYMDSPNLLTETFQSRSNPNLFFAG
QMTGVEGYVESAASGLVAGINAARLFKREEALIFPQTTAIGSLPHYVTHADSKHFQPMNVNFGIIKELEG
PRIRDKKERYEAIASRALADLDTCLASL

**SEQ ID NO: 42**

TTGTCTCAATCAACTGCAACTTATATTAATGTTATTGGAGCTGGGCTAGCTGGTTCTGAAGCTGCCTATC

AGATTGCTAAGCGCGGTATCCCCGTTAAATTGTATGAAATGCGTGGTGTCAAAGCAACACCGCAACATAA
AACCACTAATTTTGCCGAATTGGTCTGTTCCAACTCATTTCGTGGTGATAGCTTAACCAATGCAGTCGGT
CTTCTCAAAGAAGAAATGCGGCGATTAGACTCCATTATTATGCGTAATGGTGAAGCTAACCGCGTACCTG
CTGGGGGAGCAATGGCTGTTGACCGTGAGGGGTATGCAGAGAGTGTCACTGCAGAGTTGGAAAATCATCC
TCTCATTGAGGTCATTCGTGGTGAAATTACAGAAATCCCTGACGATGCTATCACGGTTATCGCGACGGGA
CCGCTGACTTCGGATGCCCTGGCAGAAAAAATTCACGCGCTAAATGGTGGCGACGGATTCTATTTTTACG
ATGCAGCAGCGCCTATCATTGATAAATCTACCATTGATATGAGCAAGGTTTACCTTAAATCTCGCTACGA
TAAAGGCGAAGCTGCTTACCTCAACTGCCCTATGACCAAAGAAGAATTCATGGCTTTCCATGAAGCTCTG
ACAACCGCAGAAGAAGCCCCGCTGAATGCCTTTGAAAAAGAAAAGTATTTTGAAGGCTGTATGCCGATTG
AAGTTATGGCTAAACGTGGCATTAAAACCATGCTTTATGGACCTATGAAACCCGTTGGATTGGAATATCC
AGATGACTATACAGGTCCTCGCGATGGAGAATTTAAAACGCCATATGCCGTCGTGCAATTGCGTCAAGAT
AATGCAGCTGGAAGCCTTTATAATATCGTTGGTTTCCAAACCCATCTCAAATGGGGTGAGCAAAAACGCG
TTTTCCAAATGATTCCAGGGCTTGAAAATGCTGAGTTTGTCCGCTACGGCGTCATGCATCGCAATTCCTA
TATGGATTCACCAAATCTTTTAACCGAAACCTTCCAATCTCGGAGCAATCCAAACCTTTTCTTTGCAGGT
CAGATGACTGGAGTTGAAGGTTATGTCGAATCAGCTGCTTCAGGTTTAGTAGCAGGAATCAATGCTGCTC
GTTTGTTCAAAAGAGAAGAAGCACTTATTTTTCCTCAGACAACAGCTATTGGGAGTTTGCCTCATTATGT
GACTCATGCCGACAGTAAGCATTTCCAACCAATGAACGTCAACTTTGGCATCATCAAAGAGTTAGAAGGC
CCACGCATTCGTGACAAAAAAGAACGTTATGAAGCTATTGCTAGTCGTGCTTTGGCAGATTTAGACACCT
GCTTAGCGTCGCTTTAA

Preferred GAS 294 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 41; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 41, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 294 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 41. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 41. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 41. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(22) GAS 253*

GAS 253 corresponds to M1 GenBank accession numbers GI:13622611, GI:15675423, and GI:21362716, to M3 GenBank accession number GI: 21910711, to M18 GenBank accession number GI: 19746473 and is also referred to as 'Spy1524' (M1), 'SpyM3_1175' (M3), 'SpyM18_1541' (M18) and 'murG'. GAS 253 has also been identified as a putative undecaprenyl-PP-MurNAc-pentapeptide-UDPGlcNAc GlcNAc transferase. Amino acid and polynucleotide sequences of GAS 253 of an M1 strain are set forth below:

**SEQ ID NO: 43**

MPKKILFTGGGTVGHVTLNLILIPKFIKDGWEVHYIGDKNGIEHTEIEKSGLDVTFHAIATGKLRRYFSW
QNLADVFKVALGLLQSLFIVAKLRPQALFSKGGFVSVPPVVAAKLLGKPVFIHESDRSMGLANKIAYKFA
TTMYTTFEQEDQLSKVKHLGAVTKVFKDANQMPESTQLEAVKEYFSRDLKTLLFIGGSAGAHVFNQFISD
HPELKQRYNIINITGDPHLNELSSHLYRVDYVTDLYQPLMAMADLVVTRGGSNTLFELLAMAKLHLIVPL
GKEASRGDQLENATYFEKRGYAKQLQEPDLTLHNFDQAMADLFEHQADYEATMLATKEIQSPDFFYDLLR
ADISSAIKEK

**SEQ ID NO: 44**

ATGCCTAAGAAGATTTTATTTACAGGTGGTGGAACTGTAGGTCATGTCACCTTGAACCTCATTCTCATAC
CAAAATTTATCAAGGACGGTTGGGAAGTACATTATATTGGTGATAAAAATGGCATTGAACATACAGAAAT

TGAAAAGTCAGGCCTTGACGTGACCTTTCATGCTATCGCGACAGGCAAGCTTAGACGCTATTTTTCATGG
CAAAATCTAGCTGATGTTTTTAAGGTTGCACTTGGCCTCCTACAGTCTCTCTTTATTGTTGCCAAGCTTC
GCCCTCAAGCCCTTTTTTCCAAAGGTGGTTTTGTCTCAGTACCGCCAGTTGTGGCTGCTAAATTGCTTGG
TAAACCAGTCTTTATTCATGAATCAGATCGGTCAATGGGACTAGCAAACAAGATTGCCTACAAATTTGCA
ACTACCATGTATACCACTTTTGAGCAGGAAGACCAGTTGTCTAAAGTTAAACACCTTGGAGCGGTGACAA
AGGTTTTCAAAGATGCCAACCAAATGCCTGAATCAACTCAGTTAGAGGCGGTGAAAGAGTATTTTAGTAG
AGACCTAAAAACCCTCTTGTTTATTGGTGGTTCGGCAGGGGCGCATGTGTTTAATCAGTTTATTAGTGAT
CATCCAGAATTGAAGCAACGTTATAATATCATCAATATTACAGGAGACCCTCACCTTAATGAATTGAGTT
CTCATCTGTATCGAGTAGATTATGTTACCGATCTCTACCAACCTTTGATGGCGATGGCTGACCTTGTAGT
GACAAGAGGGGGCTCTAATACACTTTTTGAGCTACTGGCAATGGCTAAGCTACACCTCATCGTTCCTCTT
GGTAAAGAAGCTAGCCGTGGCGATCAGTTAGAAAATGCCACTTATTTTGAGAAGAGGGGCTACGCTAAAC
AATTACAGGAACCTGATTTAACTTTGCATAATTTTGATCAGGCAATGGCTGATTTGTTTGAACATCAGGC
TGATTATGAGGCTACTATGTTGGCAACTAAGGAGATTCAGTCACCGGACTTCTTTTATGACCTTTTGAGA
GCTGATATTAGCTCCGCGATTAAGGAGAAGTAA

Preferred GAS 253 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 43; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 43, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 253 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 43. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 43. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 43. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(23) GAS 529*

GAS 529 corresponds to M1 GenBank accession numbers GI:13622403, GI:15675233, and GI:21759132, to M3 GenBank accession number GI: 21910446, to M18 GenBank accession number GI: 19746203 and is also referred to as 'Spy1280' (M1), 'SpyM3_0910' (M3), 'SpyM18_1228' (M18) and 'glmS'. GAS 529 has also been identified as a putative L-glutamine-D-fructose-6-phosphate aminotransferase (Glucosamine-6-phophate synthase). Amino acid and polynucleotide sequences of GAS 529 of an M1 strain are set forth below:

**SEQ ID NO: 45**

MCGIVGVVGNRNATDILMQGLEKLEYRGYDSAGIFVANANQTNLIKSVGRIADLRAKIGIDVAGSTGIGH
TRWATHGQSTEDNAHPHTSQTGRFVLVHNGVIENYLHIKTEFLAGHDFKGQTDTEIAVHLIGKFVEEDKL
SVLEAFKKSLSIIEGSYAFALMDSQATDTIYVAKNKSPLLIGLGEGYNMVCSDAMAMIRETSEFMEIHDK
ELVILTKDKVTVTDYDGKELIRDSYTAELDLSDIGKGTYPFYMLKEIDEQPTVMRQLISTYADETGNVQV
DPAIITSIQEADRLYILAAGTSYHAGFATKNMLEQLTDTPVELGVASEWGYHMPLLSKKPMFILLSQSGE
TADSRQVLVKANAMGIPSLTVTNVPGSTLSREATYTMLIHAGPEIAVASTKAYTAQIAALAFLAKAVGEA
NGKQEALDFNLVHELSLVAQSIEATLSEKDLVAEKVQALLATTRNAFYIGRGNDYYVAMEAALKLKEISY
IQCEGFAAGELKHGTISLIEEDTPVIALISSSQLVASHTRGNIQEVAARGAHVLTVVEEGLDREGDDIIV
NKVHPFLAPIAMVIPTQLIAYYASLQRGLDVDKPRNLAKAVTVE

**SEQ ID NO: 46**

ATGTGTGGAATTGTTGGAGTTGTTGGAAATCGCAATGCAACGGATATTTTAATGCAAGGCCTTGAAAAGC
TTGAATACCGGGGTTATGATTCAGCAGGAATTTTTGTGGCTAATGCCAATCAAACAAACTTGATTAAATC
AGTGGGGCGGATTGCTGATTTGCGTGCCAAGATTGGCATTGATGTTGCTGGTTCAACAGGGATTGGTCAC
ACCCGTTGGGCAACGCATGGCCAATCAACAGAGGATAATGCCCATCCTCACACGTCACAAACTGGACGTT

TTGTACTTGTTCATAATGGTGTGATTGAAAATTACCTTCACATTAAAACAGAGTTCCTAGCTGGACATGA
TTTTAAGGGGCAGACAGATACTGAGATTGCAGTACACTTGATTGGAAAATTTGTGGAAGAAGACAAGTTG
TCAGTACTGGAAGCTTTTAAAAAATCTTTAAGCATTATTGAAGGTTCCTACGCCTTTGCATTAATGGATA
GCCAAGCAACTGATACTATTTATGTGGCTAAAAACAAGTCTCCATTGTTGATTGGACTTGGTGAAGGTTA
CAACATGGTTTGTTCAGATGCCATGGCCATGATTCGTGAAACCAGTGAATTTATGGAAATTCATGATAAG
GAGCTAGTTATTTTAACCAAAGATAAGGTAACTGTTACAGACTACGATGGTAAAGAGCTGATACGAGATT
CCTACACTGCTGAATTAGACTTATCTGATATTGGCAAAGGGACTTATCCTTTCTATATGCTGAAAGAAAT
TGATGAGCAACCAACCGTAATGCGTCAATTAATTTCAACTTATGCAGATGAAACTGGTAACGTACAGGTT
GATCCGGCTATCATTACCTCTATCCAAGAGGCTGACCGTCTTTATATTTTAGCGGCAGGGACTTCCTACC
ATGCTGGTTTTGCAACAAAAAATATGCTTGAGCAATTGACAGATACACCAGTTGAGTTGGGCGTGGCTTC
TGAGTGGGGTTACCACATGCCTCTGCTTAGCAAGAAACCAATGTTTATTCTACTAAGCCAATCAGGAGAA
ACCGCAGATAGTCGTCAAGTTTTAGTAAAGGCAAATGCTATGGGCATTCCGAGTTTGACAGTAACTAACG
TTCCAGGATCAACCTTATCACGTGAAGCAACATACACCATGTTGATTCATGCTGGACCTGAAATTGCTGT
TGCGTCTACAAAAGCTTACACTGCACAAATTGCTGCCCTTGCCTTTTTGGCTAAGGCAGTTGGTGAGGCA
AATGGTAAGCAAGAAGCTCTTGACTTTAACTTGGTACATGAGTTGTCATTGGTTGCCCAATCTATTGAGG
CGACTTTGTCTGAAAAAGATCTCGTGGCAGAAAAGGTTCAAGCTTTGCTAGCTACTACTCGTAATGCTTT
TTACATCGGGCGTGGCAATGATTATTACGTTGCGATGGAAGCTGCTTTGAAATTAAAAGAGATTTCTTAT
ATTCAATGCGAAGGCTTTGCGGCTGGTGAATTGAAACATGGAACCATTTCATTAATTGAGGAGGACACGC
CAGTAATCGCTTTAATATCGTCTAGTCAGTTGGTTGCCTCTCATACGCGTGGTAATATTCAAGAAGTTGC
TGCCCGTGGGGCTCATGTTTTAACAGTTGTGGAAGAAGGGCTTGACCGTGAGGGAGATGACATTATTGTC
AATAAGGTTCATCCTTTCCTAGCCCCGATTGCTATGGTCATTCCAACTCAACTGATTGCTTACTACGCTT
CATTACAACGTGGACTTGATGTTGATAAGCCACGTAATTTGGCTAAAGCTGTAACAGTAGAATAA

Preferred GAS 529 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 45; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 45, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 529 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 45. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 45. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 45. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(24) GAS 045***

GAS 117 corresponds to M3 GenBank accession number GI: 21909751, M18 GenBank accesion number GI: 19745421 and is referred to as 'SpyM3_0215' (M3), 'SpyM18_oppA' (M18) and 'oppA'. GAS 045 has been identified as an oligopeptide permease. Amino acid and polynucleotide sequences of GAS 045 from an M1 strain are set forth below:

**SEQ ID NO: 47**

VTFMKKSKWLAAVSVAILSVSALAACGNKNASGGSEATKTYKYVFVNDPKSLDYILTNGG
GTTDVITQMVDGLLENDEYGNLVPSLAKDWKVSKDGLTYTYTLRDGVSWYTADGEEYAPV
TAEDFVTGLKHAVDDKSDALYVVEDSIKNLKAYQNGEVDFKEVGVKALDDKTVQYTLNKP
ESYWNSKTTYSVLFPVNAKFLKSKGKDFGTTDPSSILVNGAYFLSAFTSKSSMEFHKNEN
YWDAKNVGIESVKLTYSDGSDPGSFYKNFDKGEFSVARLYPNDPTYKSAKKNYADNITYG
MLTGDIRHLTWNLNRTSFKNTKKDPAQQDAGKKALNNKDFRQAIQFAFDRASFQAQTAGQ
DAKTKALRNMLVPPTFVTIGESDFGSEVEKEMAKLGDEWKDVNLADAQDGFYNPEKAKAE
FAKAKEALTAEGVTFPVQLDYPVDQANAATVQEAQSFKQSVEASLGKENVIVNVLETETS
THEAQGFYAETPEQQDYDIISSWWGPDYQDPRTYLDIMSPVGGGSVIQKLGIKAGQNKDV
VAAAGLDTYQTLLDEAAAITDDNDARYKAYAKAQAYLTDNAVDIPVVALGGTPRVTKAVP
PSGGPSWAGSKGPLAYKGMKLQDKPVTVKQYEKAKEKWMKAKAKSNAKYAEKLADHVEK

**SEQ ID NO: 48**

GTGACTTTTATGAAGAAAAGTAAATGGTTGGCAGCTGTAAGTGTTGCGATCTTGTCAGTA
TCCGCTTTGGCAGCTTGTGGTAATAAAAATGCTTCAGGTGGCTCAGAAGCTACAAAAACC
TACAAGTACGTTTTTGTTAACGATCCAAAATCATTGGATTATATTTTGACTAATGGCGGT
GGAACGACTGATGTGATAACACAAATGGTTGATGGTCTTTTGGAAAACGATGAGTATGGT
AATTTAGTACCATCACTTGCTAAAGATTGGAAGGTTTCAAAAGACGGTCTGACTTATACT
TATACTCTTCGCGATGGTGTCTCTTGGTATACGGCTGATGGTGAAGAATATGCCCCAGTA
ACAGCAGAAGATTTTGTGACTGGTTTGAAGCACGCGGTTGACGATAAATCAGATGCTCTT
TACGTTGTTGAAGATTCAATAAAAAACTTAAAGGCTTACCAAAATGGTGAAGTAGATTTT
AAAGAAGTTGGTGTCAAAGCCCTTGACGATAAAACTGTTCAGTATACTTTGAACAAGCCT
GAAAGCTACTGGAATTCAAAAACAACTTATAGTGTGCTTTTCCCAGTTAATGCGAAATTT
TTGAAGTCAAAAGGTAAAGATTTTGGTACAACCGATCCATCATCAATCCTTGTTAATGGT
GCTTACTTCTTGAGCGCCTTCACCTCAAAATCATCTATGGAATTCCATAAAAATGAAAAC
TACTGGGATGCTAAGAATGTTGGGATAGAATCTGTTAAATTGACTTACTCAGATGGTTCA
GACCCAGGTTCGTTCTACAAGAACTTTGACAAGGGTGAGTTCAGCGTTGCACGACTTTAC
CCAAATGACCCTACCTACAAATCAGCTAAGAAAAACTATGCTGATAACATTACTTACGGA
ATGTTGACTGGAGATATCCGTCATTTAACATGGAATTTGAACCGTACTTCTTTCAAAAAC
ACTAAGAAAGACCCTGCACAACAAGATGCCGGTAAGAAAGCTCTTAACAACAAGGATTTT
CGTCAAGCTATTCAGTTTGCTTTTGACCGAGCGTCATTCCAAGCACAAACTGCAGGTCAA
GATGCCAAAACAAAAGCCTTACGTAACATGCTTGTCCCACCAACATTTGTGACCATTGGA
GAAAGTGATTTTGGTTCAGAAGTTGAAAAGGAAATGGCAAAACTTGGTGATGAATGGAAA
GACGTTAACTTAGCTGATGCTCAAGATGGTTTCTATAATCCTGAAAAAGCAAAAGCTGAG
TTTGCAAAAGCCAAAGAAGCTTTAACAGCTGAAGGTGTAACCTTCCCAGTTCAATTAGAT
TACCCTGTTGACCAAGCAAACGCAGCAACTGTTCAGGAAGCCCAGTCTTTCAAACAATCT
GTTGAAGCATCTCTTGGTAAAGAGAATGTCATTGTCAATGTTCTTGAAACAGAAACATCA
ACTCACGAAGCCCAAGGCTTCTATGCTGAGACCCCAGAACAACAAGACTACGATATCATT
TCATCATGGTGGGGACCAGACTATCAAGATCCACGGACCTACCTTGACATCATGAGTCCA
GTAGGTGGTGGATCTGTTATCCAAAAACTTGGAATCAAAGCAGGTCAAAATAAGGATGTT
GTGGCAGCTGCAGGCCTTGATACCTACCAAACTCTTCTTGATGAAGCAGCAGCAATTACA
GACGACAACGATGCGCGCTATAAAGCTTACGCAAAAGCACAAGCCTACCTTACAGATAAT
GCCGTAGATATTCCAGTTGTGGCATTGGGTGGCACTCCACGAGTTACTAAAGCCGTTCCA
TTTAGCGGGGGCTTCTCTTGGGCAGGGTCTAAAGGTCCTCTAGCATATAAAGGAATGAAA
CTTCAAGACAAACCTGTCACAGTAAAACAATACGAAAAAGCAAAAGAAAAATGGATGAAA
GCAAAGGCTAAGTCAAATGCAAAATATGCTGAGAAGTTAGCTGATCACGTTGAAAAA

Preferred GAS 045 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 47; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 47, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 045 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 47. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 47. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 47. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 47 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(25) GAS 095*

GAS 095 corresponds to M1 GenBank accession numbers GI:13622787 and GI:15675582, to M3 GenBank accession number GI: 21911042, to M18 GenBank accession number GI: 19746634 and is

also referred to as 'Spy1733' (M1), 'SpyM3_1506' (M3), 'SpyM18_1741' (M18). GAS 095 has also been identified as a putative transcription regulator. Amino acid and polynucleotide sequences of GAS 095 of an M1 strain are set forth below:

**SEQ ID NO: 49**

MKIGKKIVLMFTAIVLTTVLALGVYLTSAYTFSTGELSKTFKDFSTSSNKSDAIKQTRAFSILLMGVDTG
SSERASKWEGNSDSMILVTVNPKTKKTTMTSLERDTLTTLSGPKNNEMNGVEAKLNAAYAAGGAQMAIMT
VQDLLNITIDNYVQINMQGLIDLVNAVGGITVTNEFDFPISIAENEPEYQATVAPGTHKINGEQALVYAR
MRYDDPEGDYGRQKRQREVIQKVLKKILALDSISSYRKILSAVSSNMQTNIEISSRTIPSLLGYRDALRT
IKTYQLKGEDATLSDGGSYQIVTSNHLLEIQNRIRTELGLHKVNQLKTNATVYENLYGSTKSQTVNNNYD
SSGQAPSYSDSHSSYANYSSGVDTGQSASTDQDSTASSHRPATPSSSSDALAADESSSSGSGSLVPPANI
NPQT

**SEQ ID NO: 50**

ATGAAAATTGGAAAAAAAATAGTTTTAATGTTCACAGCTATTGTGTTAACAACTGTCTTGGCATTAGGTG
TCTATCTAACTAGTGCTTATACCTTCTCAACAGGAGAATTATCAAAGACCTTTAAAGATTTTTCGACATC
TTCAAACAAAAGTGATGCCATTAAACAAACAAGAGCTTTTTCTATCTTGTTGATGGGTGTTGATACAGGC
TCTTCAGAGCGTGCCTCCAAGTGGGAAGGAAACAGTGATTCGATGATTTTGGTTACGGTTAATCCAAAGA
CCAAGAAAACAACTATGACTAGTTTAGAACGAGATACCTTAACCACGTTATCTGGACCCAAAAATAATGA
AATGAATGGTGTTGAAGCTAAGCTTAACGCTGCTTATGCAGCAGGTGGCGCTCAGATGGCTATTATGACC
GTGCAAGATCTTTTGAATATCACCATTGATAACTATGTTCAAATTAATATGCAAGGCCTTATTGATCTTG
TGAATGCAGTTGGAGGGATTACAGTTACAAATGAGTTTGATTTTCCTATCTCGATTGCTGAAAACGAACC
TGAATATCAAGCTACTGTTGCGCCTGGAACACACAAAATTAACGGTGAACAAGCTTTGGTTTATGCTCGT
ATGCGTTATGATGATCCTGAGGGAGATTATGGTCGACAAAAGCGTCAACGTGAAGTCATTCAAAAGGTAT
TGAAAAAAATCCTTGCTCTTGATAGCATTAGCTCTTATCGGAAGATTTTATCTGCTGTAAGTAGTAATAT
GCAAACGAATATCGAAATCTCTTCTCGCACTATCCCTAGTCTATTAGGTTATCGTGACGCACTTAGAACT
ATTAAGACTTATCAACTAAAAGGAGAAGATGCCACTTTATCAGATGGTGGATCATACCAAATTGTTACCT
CTAATCATTTGTTAGAAATCCAAAATCGTATCCGAACAGAATTAGGACTTCATAAGGTTAATCAATTAAA
AACAAATGCTACTGTTTATGAAAATTTGTATGGGTCAACTAAGTCTCAGACAGTAAACAACAACTATGAC
TCTTCAGGCCAGGCTCCATCTTATTCTGATAGTCATAGCTCTTACGCTAATTATTCAAGTGGAGTAGATA
CCGGCCAGAGTGCTAGTACAGACCAGGACTCTACTGCTTCAAGCCATAGGCCAGCTACGCCGTCTTCTTC
ATCAGATGCTTTAGCAGCTGATGAGTCTAGCTCATCAGGGTCTGGATCATTAGTTCCTCCTGCTAATATC
AACCCTCAGACCTAA

Preferred GAS 095 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 49; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 49, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 095 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 49. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 49. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 49. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 49 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(26) GAS 193*

GAS 193 corresponds to M1 GenBank accession numbers GI:13623029 and GI:15675802, to M3 GenBank accession number GI: 21911267, to M18 GenBank accession number GI: 19746914 and is

also referred to as 'Spy2025' (M1), 'SpyM3_1731' (M3), 'SpyM18_2082' (M18) and 'isp'. GAS 193 has also been identified as an immunogenic secreted protein precursor. Amino acid and polynucleotide sequences of GAS 193 of an M1 strain are set forth below:

**SEQ ID NO: 51**

MKKRKLLAVTLLSTILLNSAVPLVVADTSLRNSTSSTDQPTTADTDTDDESETPKKDKKSKETASQHDTQ
KDHKPSHTHPTPPSNDTKQTDQASSEATDKPNKDKNDTKQPDSSDQSTPSPKDQSSQKESQNKDGRPTPS
PDQQKDQTPDKTPEKSADKTPEKGPEKATDKTPEPNRDAPKPIQPPLAAAPVFIPWRESDKDLSKLKPSS
RSSAAYVRHWTGDSAYTHNLLSRRYGITAEQLDGFLNSLGIHYDKERLNGKRLLEWEKLTGLDVRAIVAI
AMAESSLGTQGVAKEKGANMFGYGAFDFNPNNAKKYSDEVAIRHMVEDTIIANKNQTFERQDLKAKKWSL
GQLDTLIDGGVYFTDTSGSGQRRADIMTKLDQWIDDHGSTPEIPEHLKITSGTQFSEVPVGYKRSQPQNV
LTYKSETYSFGQCTWYAYNRVKELGYQVDRYMGNGGDWQRKPGFVTTHKPKVGYVVSFAPGQAGADATYG
HVAVVEQIKEDGSILISESNVMGLGTISYRTFTAEQASLLTYVVGDKLPRP

**SEQ ID NO: 52**

ATGAAGAAAAGGAAATTGTTAGCAGTAACACTATTAAGTACCATACTCTTAAACAGTGCAGTGCCATTAG
TTGTTGCTGATACCTCCTTGCGTAATAGCACATCATCCACTGATCAGCCTACTACAGCAGATACTGATAC
GGATGACGAGAGTGAAACACCAAAAAAAGACAAAAAAAGCAAGGAAACAGCGTCGCAGCACGACACCCAA
AAAGACCATAAGCCATCACACACTCACCCAACCCCCCCTTCAAATGATACTAAGCAGACCGATCAGGCAT
CATCTGAAGCTACTGACAAACCAAATAAAGACAAAAACGACACCAAGCAACCAGACAGCAGTGATCAATC
CACCCCATCTCCCAAAGACCAGTCGTCTCAAAAAGAGTCACAAAACAAAGACGGCCGACCTACCCCATCA
CCTGATCAGCAAAAAGATCAGACACCTGATAAAACACCAGAAAAATCAGCTGATAAAACCCCTGAAAAAG
GACCAGAAAAAGCAACTGATAAAACACCAGAGCCAAATCGTGACGCTCCAAAACCCATCCAACCTCCTTT
AGCAGCTGCTCCTGTCTTTATACCTTGGAGAGAAAGTGACAAAGACCTGAGCAAGCTAAAACCAAGCAGT
CGCTCATCAGCGGCTTACGTGAGACACTGGACAGGTGACTCTGCCTACACTCACAACCTGTTGTCACGCC
GTTATGGGATTACTGCTGAACAGCTAGATGGTTTTTTGAACAGTCTAGGTATTCACTATGATAAAGAACG
CTTAAACGGAAAGCGTTTATTAGAATGGGAAAAACTAACAGGACTAGACGTTCGAGCTATCGTAGCTATT
GCAATGGCAGAAAGCTCACTAGGTACTCAGGGAGTTGCTAAAGAAAAAGGAGCCAATATGTTTGGTTATG
GCGCCTTTGACTTCAACCCAAACAATGCCAAAAAATACAGCGATGAGGTTGCTATTCGTCACATGGTAGA
AGACACCATCATTGCCAACAAAAACCAAACCTTTGAAAGACAAGACCTCAAAGCAAAAAAATGGTCACTA
GGCCAGTTGGATACCTTGATTGATGGTGGGGTTTACTTTACAGATACAAGTGGCAGTGGGCAAAGACGAG
CAGATATCATGACCAAACTAGACCAATGGATAGATGATCATGGAAGCACACCTGAGATTCCAGAACATCT
CAAGATAACTTCCGGGACACAATTTAGCGAAGTGCCCGTAGGTTATAAAAGAAGTCAGCCACAAAACGTT
TTGACCTACAAGTCAGAGACCTACAGCTTTGGCCAATGCACTTGGTACGCCTATAATCGTGTCAAAGAGC
TAGGTTATCAAGTCGACAGGTACATGGGTAACGGTGGCGACTGGCAGCGCAAGCCAGGTTTTGTGACCAC
CCATAAACCTAAAGTGGGCTATGTCGTCTCATTTGCACCAGGCCAAGCAGGAGCAGATGCAACCTATGGT
CACGTTGCTGTTGTAGAGCAAATCAAAGAAGATGGTTCTATCTTAATTTCAGAGTCAAATGTTATGGGAC
TAGGCACCATTTCCTATCGGACGTTCACAGCTGAGCAGGCTAGTTTGTTGACCTATGTCGTAGGGGACAA
ACTCCCAAGACCATAA

Preferred GAS 193 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 51; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 51, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 193 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 51. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 51. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 51. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(27) GAS 137*

GAS 137 corresponds to M1 GenBank accession numbers GI:13621842, GI:15674720 and GI:30173478, to M3 GenBank accession number GI:21909998, to M18 GenBank accession number GI: 19745749 and is also referred to as 'Spy0652' (M1), 'SpyM3_0462', and 'SpyM18_0713' (M18). Amino acid and polynucleotide sequences of GAS 137 of an M1 strain are set forth below:

**SEQ ID NO: 53**

MSDKHINLVIVTGMSGAGKTVAIQSFEDLGYFTIDNMPPALVPKFLELIEQTNENRRVALVVDMRSRLFF
KEINSTLDSIESNPSIDFRILFLDATDGELVSRYKETRRSHPLAADGRVLDGIRLERELLSPLKSMSQHV
VDTTKLTPRQLRKTISDQFSEGSNQASFRIEVMSFGFKYGLPLDADLVFDVRFLPNPYYQVELREKTGLD
EDVFNYVMSHPESEVFYKHLLNLIVPILPAYQKEGKSVLTVAIGCTGGQHRSVAFAHCLAESLATDWSVN
ESHRDQNRRKETVNRS

**SEQ ID NO: 54**

ATGTCAGACAAACACATTAATTTAGTTATTGTGACAGGAATGAGCGGCGCTGGAAAAACAGTTGCCATTC
AGTCTTTTGAGGATCTAGGCTACTTTACCATTGATAATATGCCCCCAGCCTTGGTTCCAAAATTTTTAGA
ATTAATTGAACAAACCAATGAAAATCGTAGGGTGGCTTTGGTTGTCGATATGAGAAGTCGTTTGTTTTTC
AAGGAAATTAATTCTACCTTAGATAGTATTGAAAGCAATCCTAGCATTGATTTTCGGATTCTTTTTTTGG
ATGCAACGGATGGAGAATTGGTGTCACGCTATAAAGAAACCAGACGGAGCCACCCTTTGGCTGCGGACGG
TCGTGTGCTTGATGGTATTCGATTGGAAAGAGAACTCCTATCTCCTTTGAAAAGCATGAGCCAACATGTG
GTGGATACAACAAAATTGACCCCTAGACAATTGCGTAAAACCATTTCAGACCAGTTTTCTGAAGGGTCTA
ATCAAGCCTCTTTCCGTATTGAAGTGATGAGCTTTGGGTTCAAATATGGTCTTCCTTTGGATGCGGATTT
GGTTTTTGATGTGCGTTTTCTACCCAATCCTTATTATCAGGTAGAGCTTCGTGAAAAAACAGGACTAGAT
GAGGACGTTTTTAATTATGTGATGTCTCACCCAGAATCAGAGGTGTTTTACAAGCATTTGTTAAACCTTA
TTGTCCCTATCTTACCGGCTTACCAAAAAGAAGGGAAGTCTGTCTTGACGGTGGCTATTGGCTGCACAGG
AGGCCAACACCGCAGCGTTGCCTTTGCCCATTGCTTGGCAGAAAGTCTGGCAACAGATTGGTCGGTTAAT
GAAAGCCATCGTGATCAAAATCGTCGTAAGGAAACGGTGAATCGTTCATGA

Preferred GAS 137 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 53; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 53, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 137 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 53. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 53. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 53. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(28) GAS 084*

GAS 084 corresponds to M1 GenBank accession numbers GI:13622398 and GI:15675229, to M3 GenBank accession number GI: 21910442, to M18 GenBank accession number GI: 19746199 and is also referred to as 'Spy1274' (M1), 'SpyM3_0906' and 'SpyM18_1223' (M18). GAS 084 has also been identified as a putative amino acid ABC transporter/periplasmic amino acid binding protein. Amino acid and polynucleotide sequences of GAS 084 of an M1 strain are set forth below:

**SEQ ID NO: 55**

MIIKKRTVAILAIASSFFLVACQATKSLKSGDAWGVYQKQKSITVGFDNTFVPMGYKDESGRCKGFDIDL

AKEVFHQYGLKVNFQAINWDMKEAELNNGKIDVIWNGYSITKERQDKVAFTDSYMRNEQIIVVKKRSDIK
TISDMKHKVLGAQSASSGYDSLLRTPKLLKDFIKNKDANQYETFTQAFIDLKSDRIDGILIDKVYANYYL
AKEGQLENYRMIPTTFENEAFSVGLRKEDKTLQAKINRAFRVLYQNGKFQAISEKWFGDDVATANIKS

**SEQ ID NO: 55**

ATGATTATAAAAAAAAGAACCGTAGCAATTTTAGCCATAGCTAGTAGCTTTTTCTTGGTAGCTTGTCAAG
CTACTAAAAGTCTTAAATCAGGAGATGCTTGGGGAGTTTACCAAAAGCAAAAAAGTATTACAGTTGGTTT
TGACAATACGTTTGTTCCTATGGGCTATAAGGATGAAAGCGGCAGATGCAAAGGTTTTGATATTGATTTG
GCTAAAGAAGTTTTTCACCAATATGGACTCAAGGTTAACTTTCAAGCTATTAATTGGGACATGAAAGAAG
CAGAACTAAACAATGGTAAAATTGATGTAATCTGGAATGGTTATTCAATAACTAAGGAGCGTCAGGATAA
GGTTGCCTTTACTGATTCTTACATGAGAAATGAACAAATTATTGTTGTCAAAAAAAGATCTGATATTAAA
ACAATATCAGATATGAAACATAAAGTGTTAGGAGCACAATCAGCTTCATCAGGTTATGACTCCTTGTTAA
GAACTCCTAAACTGCTGAAAGATTTTATTAAAAATAAAGACGCTAATCAATATGAAACCTTTACACAAGC
TTTTATTGATTTAAAATCAGATCGTATCGATGGAATATTGATTGACAAAGTATATGCCAATTACTATTTA
GCAAAAGAAGGGCAATTAGAGAATTATCGGATGATCCCAACGACCTTTGAAAATGAAGCATTTTCGGTTG
GACTTAGAAAAGAAGACAAAACGTTGCAAGCAAAAATTAATCGTGCTTTCAGGGTGCTTTATCAAAATGG
CAAATTTCAAGCTATTTCTGAGAAATGGTTTGGAGATGATGTTGCCACTGCCAATATTAAATCTTAA

Preferred GAS 084 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 55; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 55, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 084 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 55. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 55. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 55. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 55 is removed. ther fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(29) GAS 384*

GAS 384 corresponds to M1 GenBank accession numbers GI:13622908 and GI:15675693, to M3 GenBank accession number GI: 21911154, to M18 GenBank accession number GI: 19746801 and is also referred to as 'Spy1874' (M1), 'SpyM3_1618' (M3), and 'SpyM18_1939' (M18). GAS 384 has also been identified as a putative glycoprotein endopeptidase. Amino acid and polynucleotide sequences of GAS 384 of an M1 strain are set forth below:

**SEQ ID NO: 57**

MKTLAFDTSNKTLSLAILDDETLLADMTLNIQKKHSVSLMPAIDFLMTCTDLKPQDLERIVVAKGPGSYT
GLRVAVATAKTLAYSLNIALVGISSLYALAASTCKQYPNTLVVPLIDARRQNAYVGYYRQGKSVMPQAHA
SLEVIIEQLVEEGQLIFVGETAPFAEKIQKKLPQAILLPTLPSAYECGLLGQSLAPENVDAFVPQYLKRV
EAEENWLKDNEIKDDSHYVKRI

**SEQ ID NO: 58**

ATGAAGACACTTGCATTTGATACCTCAAATAAAACCTTGTCCCTTGCTATACTTGATGATGAGACACTTC
TAGCAGATATGACCCTTAACATTCAGAAAAAACATAGTGTTAGCCTTATGCCTGCTATTGATTTTTTGAT
GACTTGTACTGATCTTAAACCTCAAGATTTAGAAAGAATAGTGGTTGCAAAAGGCCCTGGATCTTACACA
GGTTTACGAGTGGCAGTTGCTACTGCAAAAACGTTAGCGTACAGTTTAAATATTGCATTGGTCGGGATTT
CGAGTCTATATGCTTTGGCTGCGTCTACTTGTAAACAGTATCCAAATACTTTGGTGGTGCCATTGATTGA
TGCTAGAAGGCAAAATGCGTATGTAGGTTATTATCGGCAAGGAAAATCAGTGATGCCACAAGCCCATGCT

TCACTAGAAGTTATTATAGAACAATTAGTAGAAGAAGGACAGCTGATTTTTGTTGGGGAGACTGCTCCTT
TTGCTGAGAAAATTCAAAAGAAACTACCTCAGGCGATACTACTTCCAACCCTTCCTTCTGCTTACGAATG
TGGTCTTTTGGGGCAAAGTTTGGCACCAGAAAATGTAGACGCCTTTGTCCCTCAATATCTCAAGAGAGTG
GAAGCTGAAGAAAACTGGCTCAAAGATAATGAGATAAAAGATGATAGTCACTACGTTAAGCGAATCTAA

Preferred GAS 384 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 57; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 57, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 384 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 57. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 57. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 57. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(30) GAS 202*

GAS 202 corresponds to M1 GenBank accession numbers GI:13622431 and GI:15675258, to M3 GenBank accession number GI: 21910527, to M18 GenBank accession number GI: 19746290 and is also referred to as 'Spy1309' (M1), 'SpyM3_0991' (M3), 'SpyM18_1321' (M18) and 'dltD'. GAS 202 has also been identified as a putative extramembranal protein. Amino acid and polynucleotide sequences of GAS 202 of an M1 strain are set forth below:

**SEQ ID NO: 59**

MLKRLWLILGPLLIAFVLVVITIFSFPTQLDHSIAQEKANAVAITDSSFKNGLIKRQALSDETCRFVPFF
GSSEWSRMDSMHPSVLAERYKRSYRPFLIGKRGSASLSHYYGIQQITNEMQKKKAIFVVSPQWFTAQGIN
PSAVQMYLSNTQVIEFLLKARTDKESQFAAKRLLELNPGVSKSNLLKKVSKGKSLSRLDRAILKCQHQVA
LREESLFSFLGKSTNYEKRILPRVKGLPKVFSYKQLNALATKRGQLATTNNRFGIKNTFYRKRIAPKYNL
YKNFQVNYSYLASPEYNDFQLLLSEFAKRKTDVLFVITPVNKAWADYTGLNQDKYQAAVRKIKFQLKSQG
FHRIADFSKDGGESYFMQDTIHLGWNGWLAFDKKVQPFLETKQPVPNYKMNPYFYSKIWANRKDLQ

**SEQ ID NO: 60**

ATGCTTAAGAGACTCTGGTTAATTCTAGGTCCTCTTCTTATTGCCTTTGTTTTAGTAGTGATTACTATTT
TTAGTTTTCCTACACAACTTGATCATTCCATAGCTCAGGAAAAAGCAAATGCCGTTGCGATCACAGATAG
TTCTTTTAAAAATGGTTTGATTAAAAGACAAGCTTTATCAGATGAGACTTGTCGTTTTGTGCCTTTTTTT
GGTTCTAGCGAATGGAGTCGAATGGATAGTATGCACCCTTCGGTGCTTGCAGAGCGCTACAAGCGGAGCT
ATAGACCATTTTTAATTGGTAAGAGAGGATCAGCATCTTTGTCGCATTATTATGGTATACAACAAATTAC
CAATGAAATGCAAAAGAAAAAAGCCATCTTTGTAGTATCTCCTCAATGGTTTACTGCTCAAGGGATTAAT
CCTAGTGCGGTTCAGATGTACTTGTCTAACACTCAAGTGATTGAATTTTTACTAAAAGCTAGAACTGATA
AAGAATCACAGTTTGCAGCAAAGCGTTTGCTTGAGCTTAACCCTGGTGTGTCTAAATCAAACTTATTGAA
AAAAGTAAGTAAGGGTAAGTCTCTTAGTCGGTTAGACAGAGCTATTTTGAAATGTCAACATCAAGTAGCA
TTGAGAGAAGAGTCCCTTTTTAGTTTTTTAGGCAAATCTACTAACTATGAAAAAAGAATTTTGCCTCGCG
TTAAGGGATTACCTAAAGTATTTTCGTATAAACAATTGAATGCATTAGCAACTAAGAGAGGCCAATTAGC
AACAACCAACAACCGTTTTGGGATTAAAAATACATTTTATCGTAAACGAATAGCACCTAAATACAATCTT
TATAAGAATTTCCAAGTTAATTATAGTTACCTGGCGTCACCAGAATACAATGATTTTCAGCTTTTATTAT
CAGAATTTGCTAAACGAAAAACAGATGTACTCTTTGTTATAACTCCTGTTAATAAAGCTTGGGCGGATTA
TACCGGCTTAAATCAAGATAAGTATCAAGCGGCAGTTCGTAAAATAAAATTCCAGTTAAAGTCACAAGGA
TTTCATCGCATTGCTGACTTCTCAAAAGATGGTGGTGAGTCCTACTTTATGCAAGATACCATCCATCTCG
GTTGGAATGGCTGGTTAGCTTTTGATAAGAAAGTGCAACCATTTCTAGAAACGAAGCAGCCAGTGCCCAA
CTATAAAATGAACCCTTATTTTTATAGTAAAATTTGGGCAAATAGGAAAGACTTGCAATAG

Preferred GAS 202 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 59; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 59, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 202 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 59. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 59. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 59. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

*(31) GAS 057*

GAS 057 corresponds to M1 GenBank accession numbers GI:13621655 and GI:15674549, to M3 GenBank accession number GI: 21909834, to M18 GenBank accession number GI: 19745560 and is also referred to as 'Spy0416' (M1), 'SpyM3_0298' (M3), 'SpyM18_0464' (M18) and 'prtS'. GAS 057 has also been identified as a putative cell envelope proteinase. Amino acid and polynucleotide sequences of GAS 057 of an M1 strain are set forth below:

**SEQ ID NO: 61**

MEKKQRFSLRKYKSGTFSVLIGSVFLVMTTTVAADELSTMSEPTITNHAQQQAQHLTNTELSSAESKSQD
TSQITLKTNREKEQSQDLVSEPTTTELADTDAASMANTGSDATQKSASLPPVNTDVHDWVKTKGAWDKGY
KGQGKVVAVIDTGIDPAHQSMRISDVSTAKVKSKEDMLARQKAAGINYGSWINDKVVFAHNYVENSDNIK
ENQFEDFDEDWENFEFDAEAEPKAIKKHKIYRPQSTQAPKETVIKTEETDGSHDIDWTQTDDDTKYESHG
MHVTGIVAGNSKEAAATGERFLGIAPEAQVMFMRVFANDIMGSAESLFIKAIEDAVALGADVINLSLGTA
NGAQLSGSKPLMEAIEKAKKAGVSVVVAAGNERVYGSDHDDPLATNPDYGLVGSPSTGRTPTSVAAINSK
WVIQRLMTVKELENRADLNHGKAIYSESVDFKDIKDSLGYDKSHQFAYVKESTDAGYNAQDVKGKIALIE
RDPNKTYDEMIALAKKHGALGVLIFNNKPGQSNRSMRLTANGMGIPSAFISHEFGKAMSQLNGNGTGSLE
FDSVVSKAPSQKGNEMNHFSNWGLTSDGYLKPDITAPGGDIYSTYNDNHYGSQTGTSMASPQIAGASLLV
KQYLEKTQPNLPKEKIADIVKNLLMSNAQIHVNPETKTTTSPRQQGAGLLNIDGAVTSGLYVTGKDNYGS
ISLGNITDTMTFDVTVHNLSNKDKTLRYDTELLTDHVDPQKGRFTLTSHSLKTYQGGEVTVPANGKVTVR
VTMDVSQFTKELTKQMPNGYYLEGFVRFRDSQDDQLNRVNIPFVGFKGQFENLAVAEESIYRLKSQGKTG
FYFDESGPKDDIYVGKHFTGLVTLGSETNVSTKTISDNGLHTLGTFKNADGKFILEKNAQGNPVLAISPN
GDNNQDFAAFKGVFLRKYQQGLKASVYHASDKEHKNPLWVSPESFKGDKNFNSDIRFAKSTTLLGTAFSGK
SLTGAELPDGHYHYVVSYYPDVVGAKRQEMTFDMILDRQKPVLSQATFDPETNRFKPEPLKDRGLAGVRK
DSVFYLERKDNKPYTVTINDSYKYVSVEDNKTFVERQADGSFILPLDKAKLGDFYYMVEDFAGNVAIAKL
GDHLPQTLGKTPIKLKLTDGNYQTKETLKDNLEMTQSDTGLVTNQAQLAVVHRNQPQSQLTKMNQDFFIS
PNEDGNKDFVAFKGLKNNVYNDLTVNVYAKDDHQKQTPIWSSQAGASVSAIESTAWYGITARGSKVMPGD
YQYVVTYRDEHGKEHQKQYTISVNDKKPMITQGRFDTINGVDHFTPDKTKALDSSGIVREEVFYLAKKNG
RKFDVTEGKDGITVSDNKVYIPKNPDGSYTISKRDGVTLSDYYYLVEDRAGNVSFATLRDLKAVGKDKAV
VNFGLDLPVPEDKQIVNFTYLVRDADGKPIENLEYYNNSGNSLILPYGKYTVELLTYDTNAAKLESDKIV
SFTLSADNNFQQVTFKITMLATSQITAHFDHLLPEGSRVSLKTAQDQLIPLEQSLYVPKAYGKTVQEGTY
EVVVSLPKGYRIEGNTKVNTLPNEVHELSLRLVKVGDASDSTGDHKVMSKNNSQALTASATPTKSTTSAT
AKALPSTGEKMGLKLRIVGLVLLGLTCVFSRKKSTKD

**SEQ ID NO: 62**

GTGGAGAAAAAGCAACGTTTTTCCCTTAGAAAATACAAATCAGGAACGTTTTCGGTCTTAATAGGAAGCG
TTTTCTTGGTGATGACAACAACAGTAGCAGCAGATGAGCTAAGCACAATGAGCGAACCAACAATCACGAA
TCACGCTCAACAACAAGCGCAACATCTCACCAATACAGAGTTGAGCTCAGCTGAATCAAAATCTCAAGAC
ACATCACAAATCACTCTCAAGACAAATCGTGAAAAAAGAGCAATCACAAGATCTAGTCTCTGAGCCAACCA
CAACTGAGCTAGCTGACACAGATGCAGCATCAATGGCTAATACAGGTTCTGATGCGACTCAAAAAAGCGC
TTCTTTACCGCCAGTCAATACAGATGTTCACGATTGGGTAAAAACCAAAGGAGCTTGGGACAAGGGATAC

AAAGGACAAGGCAAGGTTGTCGCAGTTATTGACACAGGGATCGATCCGGCCCATCAAAGCATGCGCATCA
GTGATGTATCAACTGCTAAAGTAAAATCAAAAGAAGACATGCTAGCACGCCAAAAAGCCGCCGGTATTAA
TTATGGGAGTTGGATAAATGATAAAGTTGTTTTTGCACATAATTATGTGGAAAATAGCGATAATATCAAA
GAAAATCAATTCGAGGATTTTGATGAGGACTGGGAAAACTTTGAGTTTGATGCAGAGGCAGAGCCAAAAG
CCATCAAAAAACACAAGATCTATCGTCCCCAATCAACCCAGGCACCGAAAGAAACTGTTATCAAAACAGA
AGAAACAGATGGTTCACATGATATTGACTGGACACAAACAGACGATGACACCAAATACGAGTCACACGGT
ATGCATGTGACAGGTATTGTAGCCGGTAATAGCAAAGAAGCCGCTGCTACTGGAGAACGCTTTTTAGGAA
TTGCACCAGAGGCCCAAGTCATGTTCATGCGTGTTTTTGCCAACGACATCATGGGATCAGCTGAATCACT
CTTTATCAAAGCTATCGAAGATGCCGTGGCTTTAGGAGCAGATGTGATCAACCTGAGTCTTGGAACCGCT
AATGGGGCACAGCTTAGTGGCAGCAAGCCTCTAATGGAAGCAATTGAAAAAGCTAAAAAAGCCGGTGTAT
CAGTTGTTGTAGCAGCAGGAAATGAGCGCGTCTATGGATCTGACCATGATGATCCATTGGCGACAAATCC
AGACTATGGTTTGGTCGGTTCTCCCTCAACAGGTCGAACACCAACATCAGTGGCAGCTATAAACAGTAAG
TGGGTGATTCAACGTCTAATGACGGTCAAAGAATTAGAAAACCGTGCCGATTTAAACCATGGTAAAGCCA
TCTATTCAGAGTCTGTCGACTTTAAAGACATAAAAGATAGCCTAGGTTATGATAAATCGCATCAATTTGC
TTATGTCAAAGAGTCAACTGATGCGGGTTATAACGCACAAGACGTTAAAGGTAAAATTGCTTTAATTGAA
CGTGATCCCAATAAAACCTATGACGAAATGATTGCTTTGGCTAAGAAACATGGAGCTCTGGGAGTACTTA
TTTTTAATAACAAGCCTGGTCAATCAAACCGCTCAATGCGTCTAACAGCTAATGGGATGGGGATACCATC
TGCTTTCATATCGCACGAATTTGGTAAGGCCATGTCCCAATTAAATGGCAATGGTACAGGAAGTTTAGAG
TTTGACAGTGTGGTCTCAAAAGCACCGAGTCAAAAAGGCAATGAAATGAATCATTTTTCAAATTGGGGCC
TAACTTCTGATGGCTATTTAAAACCTGACATTACTGCACCAGGTGGCGATATCTATTCTACCTATAACGA
TAACCACTATGGTAGCCAAACAGGAACAAGTATGGCCTCTCCTCAGATTGCTGGCGCCAGCCTTTTGGTC
AAACAATACCTAGAAAAGACTCAGCCAAACTTGCCAAAAGAAAAAATTGCTGATATCGTTAAGAACCTAT
TGATGAGCAATGCTCAAATTCATGTTAATCCAGAGACAAAAACGACCACCTCACCGCGTCAGCAAGGGGC
AGGATTACTTAATATTGACGGAGCTGTCACTAGCGGCCTTTATGTGACAGGAAAAGACAACTATGGCAGT
ATATCATTAGGCAACATCACAGATACGATGACGTTTGATGTGACTGTTCACAACCTAAGCAATAAAGACA
AAACATTACGTTATGACACAGAATTGCTAACAGATCATGTAGACCCACAAAAGGGCCGCTTCACTTTGAC
TTCTCACTCCTTAAAAACGTACCAAGGAGGAGAAGTTACAGTCCCAGCCAATGGAAAAGTGACTGTAAGG
GTTACCATGGATGTCTCACAGTTCACAAAAGAGCTAACAAAACAGATGCCAAATGGTTACTATCTAGAAG
GTTTTGTCCGCTTTAGAGATAGTCAAGATGACCAACTAAATAGAGTAAACATTCCTTTTGTTGGTTTTAA
AGGGCAATTTGAAAACTTAGCAGTTGCAGAAGAGTCCATTTACAGATTAAAATCTCAAGGCAAAACTGGT
TTTTACTTTGATGAATCAGGTCCAAAAGACGATATCTATGTCGGTAAACACTTTACAGGACTTGTCACTC
TTGGTTCAGAGACCAATGTGTCAACCAAAACGATTTCTGACAATGGTCTACACACACTTGGCACCTTTAA
AAATGCAGATGGCAAATTTATCTTAGAAAAAAATGCCCAAGGAAACCCTGTCTTAGCCATTTCTCCAAAT
GGTGACAACAACCAAGATTTTGCAGCCTTCAAAGGTGTTTTCTTGAGAAAATATCAAGGCTTAAAAGCAA
GTGTCTACCATGCTAGTGACAAGGAACACAAAAATCCACTGTGGGTCAGCCCAGAAAGCTTTAAAGGAGA
TAAAAACTTTAATAGTGACATTAGATTTGCAAAATCAACGACCCTGTTAGGCACAGCATTTTCTGGAAAA
TCGTTAACAGGAGCTGAATTACCAGATGGGCATTATCATTATGTGGTGTCTTATTACCCAGATGTGGTCG
GTGCCAAACGTCAAGAAATGACATTTGACATGATTTTAGACCGACAAAAACCGGTACTATCACAAGCAAC
ATTTGATCCTGAAACAAACCGATTCAAACCAGAACCCCTAAAAGACCGTGGATTAGCTGGTGTTCGCAAA
GACAGTGTCTTTTATCTAGAAAGAAAAGACAACAAGCCTTATACAGTTACGATAAACGATAGCTACAAAT
ATGTCTCAGTAGAAGACAATAAAACATTTGTGGAGCGACAAGCTGATGGCAGCTTTATCTTGCCGCTTGA
TAAAGCAAAATTAGGGGATTTCTATTACATGGTCGAGGATTTTGCAGGGAACGTGGCCATCGCTAAGTTA
GGAGATCACTTACCACAAACATTAGGTAAAACACCAATTAAACTTAAGCTTACAGACGGTAATTATCAGA
CCAAAGAAACGCTTAAAGATAATCTTGAAATGACACAGTCTGACACAGGTCTAGTCACAAATCAAGCCCA
GCTAGCAGTGGTGCACCGCAATCAGCCGCAAAGCCAGCTAACAAAGATGAATCAGGATTTCTTTATCTCA
CCAAACGAAGATGGGAATAAAGACTTTGTGGCCTTTAAAGGCTTGAAAAATAACGTGTATAATGACTTAA
CGGTTAACGTATACGCTAAAGATGACCACCAAAAACAAACCCCTATCTGGTCTAGTCAAGCAGGCGCTAG
TGTATCCGCTATTGAAAGTACAGCCTGGTATGGCATAACAGCCCGAGGAAGCAAGGTGATGCCAGGTGAT
TATCAGTATGTTGTGACCTATCGTGACGAACATGGTAAAGAACATCAAAAGCAGTACACCATATCTGTGA
ATGACAAAAAACCAATGATCACTCAGGGACGTTTTGATACCATTAATGGCGTTGACCACTTTACTCCTGA
CAAGACAAAAGCCCTTGACTCATCAGGCATTGTCCGCGAAGAAGTCTTTTACTTGGCCAAGAAAAATGGC
CGTAAATTTGATGTGACAGAAGGTAAAGATGGTATCACAGTTAGTGACAATAAGGTGTATATCCCTAAAA
ATCCAGATGGTTCTTACACCATTTCAAAAAGAGATGGTGTCACACTGTCAGATTATTACTACCTTGTCGA
AGATAGAGCTGGTAATGTGTCTTTTGCTACCTTGCGTGACCTAAAAGCGGTCGGAAAAGACAAAGCAGTA
GTCAATTTTGGATTAGACTTACCGGTCCCTGAAGACAAACAAATAGTGAACTTTACCTACCTTGTGCGGG
ATGCAGATGGTAAACCGATTGAAAACCTAGAGTATTATAATAACTCAGGTAACAGTCTTATCTTGCCATA
CGGCAAATACACGGTCGAATTGTTGACCTATGACACCAATGCAGCCAAACTAGAGTCAGATAAAATCGTT
TCCTTTACCTTGTCAGCTGATAACAACTTCCAACAAGTTACCTTTAAGATAACGATGTTAGCAACTTCTC
AAATAACTGCCCACTTTGATCATCTTTTGCCAGAAGGCAGTCGCGTTAGCCTTAAAACAGCTCAAGATCA
GCTAATCCCGCTTGAACAGTCCTTGTATGTGCCTAAAGCTTATGGCAAAACCGTTCAAGAAGGCACTTAC
GAAGTTGTTGTCAGCCTGCCTAAAGGCTACCGTATCGAAGGCAACACAAAGGTGAATACCCTACCAAATG
AAGTGCACGAACTATCATTACGCCTTGTCAAAGTAGGAGATGCCTCAGATTCAACTGGTGATCATAAGGT

TATGTCAAAAAATAATTCACAGGCTTTGACAGCCTCTGCCACACCAACCAAGTCAACGACCTCAGCAACA
GCAAAAGCCCTACCATCAACGGGTGAAAAAATGGGTCTCAAGTTGCGCATAGTAGGTCTTGTGTTACTCG
GACTTACTTGCGTCTTTAGCCGAAAAAAATCAACCAAAGATTGA

Preferred GAS 057 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 61; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 61, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 057 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 61. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 61. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 61. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 61 is removed. In another example, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 61 is removed. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The immunogenicity of other known GAS antigens may be improved by combination with two or more GAS the first antigen group. Such other known GAS antigens include a second antigen group consisting of (1) one or more variants of the M surface protein or fragments thereof, (2) fibronectin-binding protein, (3) streptococcal heme-associated protein, or (4) SagA. These antigens are referred to herein as the "second antigen group".

The invention thus includes an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group and one, two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

Each of the GAS antigens of the second antigen group are described in more detail below.

***(1) M surface protein***

Over 100 different type variants of the M protein have been identified. Epitopes having increased bactericidal activity and having decreased likelihood of cross-reacting with human tissues have been identified in the amino terminal region and combined into fusion proteins containing approximately six, seven, or eight M protein fragments linked in tandem. See Ref. 4, 5, 6, WO 02/094851 and WO 94/06465. (Each of the M protein variants, fragments and fusion proteins described in these references are specifically incorporated herein by reference.)

Accordingly, the compositions of the invention may further comprise a GAS M surface protein or a fragment or derivative thereof. One or more GAS M surface protein fragments may be combined together in a fusion protein. Alternatively, one or more GAS M surface protein fragments are combined with a GAS antigen or fragment thereof of the first antigen group. One example of a GAS M protein is set forth below.

**SEQ ID NO: 63**

MAKNNTNRHYSLRKLKTGTASVAVALTVLGAGFANQTEVKANGDGNPREVIEDLAANNPAIQNIRLRYEN
KDLKARLENAMEVAGRDFKRAEELEKAKQALEDQRKDLETKLKELQQDYDLAKESTSWDRQRLEKELEEK
KEALELAIDQASRDYHRATALEKELEEKKKALELAIDQASQDYNRANVLEKELETITREQEINRNLLGNA
KLELDQLSSEKEQLTIEKAKLEEEKQISDASRQSLRRDLDASREAKKQVEKDLANLTAELDKVKEDKQIS
DASRQGLRRDLDASREAKKQVEKDLANLTAELDKVKEEKQISDASRQGLRRDLDASREAKKQVEKALEEA
NSKLAALEKLNKELEESKKLTEKEKAELQAKLEAEAKALKEQLAKQAEELAKLRAGKASDSQTPDTKPGN
KAVPGKGQAPQAGTKPNQNKAPMKETKRQLPSTGETANPFFTAAALTVMATAGVAAVVKRKEEN

Preferred GAS M proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 63; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 63, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS M proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 63. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 63. Preferably, the fragment is one of those described in the references above. Preferably, the fragment is constructed in a fusion protein with one or more additional M protein fragments. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 63. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(2) Fibronectin-binding protein***

GAS fibronectin-binding protein ('SfbI') is a mutlifunctional bacterial protein thought to mediate attachment of the bacteria to host cells, facilitate bacterial internalization into cells and to bind to the Fc fragment of human IgG, thus interfering with Fc-receptor mediated phagocytosis and antibody-dependent cell cytotoxicity. Immunization of mice with SfbI and an 'H12 fragment' (encoded by positions 1240 – 1854 of the SfbI gene) are discussed in Refs. 7,8 and 9. One example of an amino acid sequence for GAS SfbI is show below.

**SEQ ID NO: 64**

MSFDGFFLHHLTNELKENLLYGRIQKVNQPFERELVLTIRNHRKNYKLLLSAHPVFGRVQITQADFQNPQ
VPNTFTMIMRKYLQGAVIEQLEQIDNDRIIEIKVSNKNEIGDAIQATLIIEIMGKHSNIILVDRAENKII
ESIKHVGFSQNSYRTILPGSTYIEPPKTAAVNPFTITDVPLPEILQTQELTVKSLQQHFQGLGRDTAKEL
AELLTTDKLKRFREFFARPTQANLTTASFAPVLFSDSHATFETLSDMLDHFYQDKAERDRINQQASDLIH
RVQTELDKNRNKLSKQEAELLATENAELFRQKGELLTTYLSLVPNNQDSVILDNYYTGEKIEIALDKALT
PNQNAQRYFKKYQKLKEAVKHLSGLIADTKQSITYFESVDYNLSQASIDDIEDIREELYQAGFLKSRQRD
KRHKRKKPEQYLASDGTTILMVGRNNLQNEELTFKMAKKGELWFHAKDIPGSHVIIKDNLDPSDEVKTDA
AELAAYYSKARLSNLVQVDMIEAKKLHKPSGAKPGFVTYTGQKTLRVTPDQAKILSMKLS

Preferred SfbI proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 64; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 64, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These SfbI proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 64. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 64. Preferably, the fragment is one of those described in the references above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 64. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(3) Streptococcal heme-associated protein***

The GAS streptococcal heme-associated protein ('Shp') has been identified as a GAS cell surface protein. It is thought to be cotrascribed with genes encoding homologues of an ABC transporter involved in iron uptake in gram-negative bacteria. The Shp protein is further described in 10. One example of a Shp protein is shown below:

**SEQ ID NO: 65**

MTKVVIKQLLQVIVVFMISLSTMTNLVYADKGQIYGCIIQRNYRHPISGQIEDSGGEHSFDIGQGMVEGT
VYSDAMLEVSDAGKIVLTFRMSLADYSGNYQFWIQPGGTGSFQAVDYNITQKGTDTNGTTLDIAISLPTV
NSIIRGSMFVEPMGREVVFYLSASELIQKYSGNMLAQLVTETDNSQNQEVKDSQKPVDTKLGESQDESHT
GAMITQNKPKANSSNNKSLSDKKILPSKMGLTTSLELKKEDKFRSKKDLSIMIYYFPTFFLMLGGFAVWV
WKKRKKNDKTM

Preferred Shp proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 65; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 65, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These Shp proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 65. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 65. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 65. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

***(4) SagA***

Streptolysin S (SLS), also known as 'SagA', is thought to be produced by almost all GAS colonies. This cytolytic toxin is responsible for the beta-hemolysis surrounding colonies of GAS grown on blood agar and is thought to be associated with virulence. While the full SagA peptide has not been

shown to be immunogenic, a fragment of amino acids 10 – 30 (SagA 10 – 30) has been used to produce neutralizing antibodies. See Ref. 11. The amino acid sequence of SagA 10 – 30 is shown below:

SEQ ID NO: 66 FSIATGSGNSQGGSGSYTPGKC

Preferred SagA 10-30 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 66; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 66, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, or 20). These SagA 10 - 30 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 66.

There is an upper limit to the number of GAS antigens which will be in the compositions of the invention. Preferably, the number of GAS antigens in a composition of the invention is less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, or less than 3. Still more preferably, the number of GAS antigens in a composition of the invention is less than 6, less than 5, or less than 4. Still more preferably, the number of GAS antigens in a composition of the invention is 3.

The GAS antigens used in the invention are preferably isolated, i.e., separate and discrete, from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

***Fusion proteins***

The GAS antigens used in the invention may be present in the composition as individual separate polypeptides, but it is preferred that at least two (*i.e.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) of the antigens are expressed as a single polypeptide chain (a 'hybrid' polypeptide). Hybrid polypeptides offer two principal advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

The hybrid polypeptide may comprise two or more polypeptide sequences from the first antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a GAS antigen or a fragment thereof of the first antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the second antigen group. Accordingly, the invention

includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a GAS antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a GAS antigen or a fragment thereof from the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes.

Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten GAS antigens are preferred. In particular, hybrids consisting of amino acid sequences from two, three, four, or five GAS antigens are preferred.

Different hybrid polypeptides may be mixed together in a single formulation. Within such combinations, a GAS antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Hybrid polypeptides can be represented by the formula **$NH_2$-A-{-X-L-}$_n$-B-COOH**, wherein: X is an amino acid sequence of a GAS antigen or a fragment thereof from the first antigen group or the second antigen group; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and *n* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of $X_1$ will be retained, but the leader peptides of $X_2$ ... $X_n$ will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of $X_1$ as moiety -A-.

For each *n* instances of {-X-L-}, linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be $NH_2$-$X_1$-$L_1$-$X_2$-$L_2$-COOH, $NH_2$-$X_1$-$X_2$-COOH, $NH_2$-$X_1$-$L_1$-$X_2$-COOH, $NH_2$-$X_1$-$X_2$-$L_2$-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising $Gly_n$ where *n* = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* $His_n$ where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG, with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* $His_n$ where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be

apparent to those skilled in the art. If $X_1$ lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* $His_n$ where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Most preferably, $n$ is 2 or 3.

The invention also provides nucleic acid encoding hybrid polypeptides of the invention. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Polypeptides of the invention can be prepared by various means (*e.g.* recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (*e.g.* native, fusions, non-glycosylated, lipidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other GAS or host cell proteins).

Nucleic acid according to the invention can be prepared in many ways (*e.g.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other GAS or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA), *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing purposes).

The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

*Strains*

Preferred polypeptides of the invention comprise an amino acid sequence found in an M1, M3 or M18 strain of GAS. The genomic sequence of an M1 GAS strain is reported at Ref. 12. The genomic sequence of an M3 GAS strain is reported at Ref. 13. The genomic sequence of an M18 GAS strain is reported at Ref. 14.

Where hybrid polypeptides are used, the individual antigens within the hybrid (*i.e.* individual -X- moieties) may be from one or more strains. Where $n$=2, for instance, $X_2$ may be from the same strain as $X_1$ or from a different strain. Where $n$=3, the strains might be (i) $X_1$=$X_2$=$X_3$ (ii) $X_1$=$X_2$≠$X_3$ (iii) $X_1$≠$X_2$=$X_3$ (iv) $X_1$≠$X_2$≠$X_3$ or (v) $X_1$=$X_3$≠$X_2$, *etc.*

***Purification and Recombinant Expression***

The GAS antigens of the invention may be isolated from a *Streptococcus pyogenes*, or they may be recombinantly produced, for instance, in a heterologous host. Preferably, the GAS antigens are prepared using a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria* (*e.g. M.tuberculosis*), yeasts, *etc.*

Recombinant production of polypeptides is facilitated by adding a tag protein to the GAS antigen to be expressed as a fusion protein comprising the tag protein and the GAS antigen. Such tag proteins can facilitate purification, detection and stability of the expressed protein. Tag proteins suitable for use in the invention include a polyarginine tag (Arg-tag), polyhistidine tag (His-tag), FLAG-tag, Strep-tag, c-myc-tag, S-tag, calmodulin-binding peptide, cellulose-binding domain, SBP-tag,, chitin-binding domain, glutathione S-transferase-tag (GST), maltose-binding protein, transcription termination anti-terminiantion factor (NusA), *E. coli* thioredoxin (TrxA) and protein disulfide isomerase I (DsbA). Preferred tag proteins include His-tag and GST. A full discussion on the use of tag proteins can be found at Ref. 15.

After purification, the tag proteins may optionally be removed from the expressed fusion protein, i.e., by specifically tailored enzymatic treatments known in the art. Commonly used proteases include enterokinase, tobacco etch virus (TEV), thrombin, and factor $X_a$.

***Immunogenic compositions and medicaments***

Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 8, preferably about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen-free. The composition may be isotonic with respect to humans.

Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of a *Streptococcus pyogenes* infection

in an animal susceptible to streptococcal infection comprising administering to said animal a therapeutic or prophylactic amount of the immunogenic compositions of the invention. Preferably, the immunogenic composition comprises a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group. Preferably, the combination of GAS antigens consists of three, four, five, six, seven, eight, nine, or ten GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens consists of three, four, or five GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117.

Alternatively, the invention includes an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group and one, two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of the compositions of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides for a kit comprising a first component comprising a combination of GAS antigens. In one embodiment, the combination of GAS antigens consists of a mixture of two to thirty-one GAS antigens selected from the first antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Preferably, the combination consists of three, four, or five GAS antigens from the first antigen group. Preferably, the combination includes either or both of GAS 117 and GAS 040.

In another embodiment, the kit comprises a first component comprising a combination of GAS antigens consisting of a mixture of two to thirty-one GAS antigens of the first antigen group and one, two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

The invention also provides a delivery device pre-filled with the immunogenic compositions of the invention.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies and/or cell-mediated immunity. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by *Streptococcus pyogenes* (*e.g.* pharyngitis (such as streptococcal sore throat), scarlet fever, impetigo, erysipelas, cellulitis, septicemia, toxic shock syndrome, necrotizing fasciitis (flesh eating disease) and sequelae (such as rheumatic fever and acute glomerulonephritis)). The compositions may also be effective against other streptococcal bacteria.

One way of checking efficacy of therapeutic treatment involves monitoring GAS infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the GAS antigens in the compositions of the invention after administration of the composition.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral (*e.g.* tablet, spray), vaginal, topical, transdermal {*e.g.* see ref. 16} or transcutaneous {*e.g.* see refs. 17 & 18}, intranasal {*e.g.* see ref. 19}, ocular, aural, pulmonary or other mucosal administration.

The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.* a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, *etc.*

The compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is

reconstituted just prior to administration to a patient. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

***Further components of the composition***

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in reference 20.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant.

Preferred further adjuvants include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphoshpates, orthophosphates), sulphates, *etc.* {*e.g.* see chapters 8 & 9 of ref. 21}), or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt. See ref. 22.

B. Oil-Emulsions

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See ref. 23.

Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

C. Saponin Formulations

Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs.

Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-LC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in U.S. Patent No. 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO 96/33739).

Combinations of saponins and cholesterols can be used to form unique particles called Immunostimulating Complexs (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP 0 109 942, WO 96/11711 and WO 96/33739. Optionally, the ISCOMS may be devoid of additional detergent. See ref. 24.

A review of the development of saponin based adjuvants can be found at ref. 25.

C. Virosomes and Virus Like Particles (VLPs)

Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO 03/024480, WO 03/024481, and Refs. 26, 27, 28 and 29. Virosomes are discussed further in, for example, Ref. 30

D. Bacterial or Microbial Derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

(1) *Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)*

Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529. See Ref. 31.

(2) *Lipid A Derivatives*

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in Ref. 32 and 33.

(3) *Immunostimulatory oligonucleotides*

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analog such as 2'-deoxy-7-deazaguanosine. See ref. 34, WO 02/26757 and WO 99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Refs. 35, 36, WO 98/40100, U.S. Patent No. 6,207,646, U.S. Patent No. 6,239,116, and U.S. Patent No. 6,429,199.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See ref. 37. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 38, 39 and WO 01/95935. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 40, 41, 42 and WO 03/035836.

(4) *ADP-ribosylating toxins and detoxified derivatives thereof.*

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (i.e., E. coli heat labile enterotoxin "LT), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO 95/17211 and as parenteral adjuvants in WO 98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63.

E. Human Immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

F. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Ref. 43) or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. E.g., ref. 44.

G. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

H. Liposomes

Examples of liposome formulations suitable for use as adjuvants are described in U.S. Patent No. 6,090,406, U.S. Patent No. 5,916,588, and EP 0 626 169.

I. Polyoxyethylene ether and Polyoxyethylene Ester Formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. Ref. 45. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (Ref. 46) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (Ref. 47).

Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in Ref. 48 and 49.

K. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues, described further in Ref. 50 and 51.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

(1) a saponin and an oil-in-water emulsion (ref. 52);

(2) a saponin (e.g.., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) (see WO 94/00153);

(3) a saponin (e.g.., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) + a cholesterol;

(4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) (Ref. 53);

combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (Ref. 54);

(5) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(6) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and

(7) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant bacterial toxins are preferred mucosal adjuvants.

The composition may include an antibiotic.

***Further antigens***

The compositions of the invention may further comprise one or more additional non-GAS antigens, including additional bacterial, viral or parasitic antigens.

In one embodiment, the GAS antigen combinations of the invention are combined with one or more additional, non-GAS antigens suitable for use in a paediatric vaccine. For example, the GAS antigen combinations may be combined with one or more antigens derived from a bacteria or virus selected from the group consisting of *N. meningitidis* (including serogroup A, B, C, W135 and/or Y), *Streptococcus pneumoniae, Bordetella pertussis, Moraxella catarrhalis, Tetanus, Diphtheria,* Respiratory Syncytial virus ('RSV'), polio, measles, mumps, rubella, and rotavirus.

In another embodiment, the GAS antigen combinations of the invention are combined with one or more additional, non-GAS antigens suitable for use in a vaccine designed to protect elderly or immunocomprised individuals. For example, the GAS antigen combinations may be combined with an antigen derived from the group consisting of *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeruginosa, Legionella pneumophila, Listeria monocytogenes*, influenza, and Parainfluenza virus ('PIV').

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity {*e.g.* refs. 55 to 64}. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The $CRM_{197}$ diphtheria toxoid is particularly preferred {65}. Other carrier polypeptides include the *N.meningitidis* outer membrane protein {66},

synthetic peptides {67, 68}, heat shock proteins {69, 70}, pertussis proteins {71, 72}, protein D from *H.influenzae* {73}, cytokines {74}, lymphokines, hormones, growth factors, toxin A or B from *C.difficile* {75}, iron-uptake proteins {76}, *etc.* Where a mixture comprises capsular saccharides from both serogroups A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary *e.g.* detoxification of pertussis toxin by chemical and/or genetic means.

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used {*e.g.* refs. 77 to 85}. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

***Definitions***

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 86. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in reference 87.

The following example demonstrates one way of preparing recombinant GAS antigens of the invention and testing their efficacy in a murine model.

**EXAMPLE 1: Preparation of recombinant GAS antigens of the invention and Demonstration of Efficacy in Murine Model.**

Recombinant GAS proteins corresponding to two or more of the GAS antigens of the first antigen group are expressed as follows.

1. Cloning of GAS antigens for expression in E. coli

The selected GAS antigens were cloned in such a way to obtain two different kinds of recombinant proteins: (1) proteins having an hexa-histidine tag at the carboxy-terminus (Gas-His) and (2) proteins having the hexa-histidine tag at the carboxy-terminus and GST at the amino-terminus (Gst-Gas-His). Type (1) proteins were obtained by cloning in a pET21b+vector (available from Novagen). The type (2) proteins were obtained by cloning in a pGEX-NNH vector. This cloning strategy allowed for the GAS genomic DNA to be used to amplify the selected genes by PCR, to perform a single restriction enzyme digestion of the PCR products and to clone then simultaneously into both vectors.

(a) *Construction of pGEX-NNH expression vectors*

Two couples of complementary oligodeoxyribonucleotides are synthesised using the DNA synthesiser ABI394 (Perkin Elmer) and reagents from Cruachem (Glasgow, Scotland). Equimolar amounts of the oligo pairs (50 ng each oligo) are annealed in T4 DNA ligase buffer (New England Biolabs) for 10 min in a final volume of 50 µl and then left to cool slowly at room temperature. With the described procedure the following DNA linkers are obtained:

**gexNN linker**

```
      NdeI  NheI XmaI  EcoRI   NcoI       SalI     XhoI       SacI
GATCCCATATGGCTAGCCCGGGGAATTCGTCCATGGAGTGAGTCGACTGACTCGAGTGATCGAGCTC
    GGTATACCGATCGGGCCCCTTAAGCAGGTACCTCACTCAGCTGACTGAGCTCACTAGCTCGAG

     NotI
CTGAGCGGCCGCATGAA
GACTCGCCGGCGTACTTTCGA
```

**gexNNH linker**

```
      HindIII   NotI'      XhoI       Hexa-Histidine
TCGACAAGCTTGCGGCCGCACTCGAGCATCACCATCACCATCACTGAT
    GTTCGAACGCCGGCGTGAGCACGTAGAGGTAGTGGTAGTGACTATCGA
```

The plasmid pGEX-KG [K. L. Guan and J. E. Dixon, *Anal. Biochem.* 192, 262 (1991)] is digested with BamHI and HindIII and 100 ng is ligated overnight at 16 °C to the linker gexNN with a molar ratio of 3:1 linker/plasmid using 200 units of T4 DNA ligase (New england Biolabs). After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NN plasmid, having the correct linker, is selected by means of restriction enzyme analysis and DNA sequencing.

The new plasmid pGEX-NN is digested with SalI and HindIII and ligated to the linker gexNNH. After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NNH plasmid, having the correct linker, is selected by means of restriction enzyme analysis and DNA sequencing.

(b) *Chromosomal DNA preparation*

GAS SF370 strain is grown in THY medium until $OD_{600}$ is 0.6-0.8. Bacteria are then centrifuged, suspended in TES buffer with lyzozyme (10mg/ml) and mutanolysine (10U/µl) and incubated 1 hr at 37° C. Following treatment of the bacterial suspension with RNAase, Proteinase K and 10% Sarcosyl/EDTA, protein extraction with saturated phenol and phenol/chloroform is carried out. The resulting supernatant is precipitated with Sodium Acetate/Ethanol and the extracted DNA is pelletted by centrifugation, suspended in Tris buffer and kept at –20° C.

(c) *Oligonucleotide design*

Synthetic oligonucleotide primers are designed on the basis of the coding sequence of each GAS antigen using the sequence of *Streptococcus pyogenes* SF370 M1 strain. Any predicted signal peptide is omitted, by deducing the 5' end amplification primer sequence immediately downstream from the predicted leader sequence. For most GAS antigens, the 5' tail of the primers (see Table 1, below) include only one restriction enzyme recognition site (NdeI, or NheI, or SpeI depending on the gene's own restriction pattern); the 3' primer tails (see Table 1) include a XhoI or a NotI or a HindIII restriction site.

| 5' tails | | 3' tails | |
|---|---|---|---|
| NdeI | 5' GTGCGTCATATG 3' | XhoI | 5' GCGTCTCGAG 3' |
| NheI | 5' GTGCGTGCTAGC 3' | NotI | 5' ACTCGCTAGCGGCCGC 3' |
| SpeI | 5' GTGCGTACTAGT 3' | HindIII | 5' GCGTAAGCTT 3' |

**Table 1.** Oligonucleotide tails of the primers used to amplify genes encoding selected GAS antigens.

As well as containing the restriction enzyme recognition sequences, the primers include nucleotides which hybridize to the sequence to be amplified. The number of hybridizing nucleotides depends on the melting temperature of the primers which can be determined as described [(Breslauer et al., Proc. Nat. Acad. Sci. 83, 3746-50 (1986 )]. The average melting temperature of the selected oligos is 50-55 °C for the hybridizing region alone and 65-75 °C for the whole oligos. Oligos can be purchased from MWG-Biotech S.p.A. (Firenze, Italy).

(d) *PCR amplification*

The standard PCR protocol is as follows: 50 ng genomic DNA are used as template in the presence of 0,2 µM each primer, 200 µM each dNTP, 1,5 mM $MgCl_2$, 1x PCR buffer minus Mg (Gibco-BRL), and 2 units of Taq DNA polymerase (Platinum Taq, Gibco-BRL) in a final volume of 100 µl. Each sample undergoes a double-step amplification: the first 5 cycles are performed using as the hybridizing temperature of one of the oligos excluding the restriction enzyme tail, followed by 25

cycles performed according to the hybridization temperature of the whole length primers. The standard cycles are as follows:

one cycle:
denaturation : 94 °C, 2 min

5 cycles:
denaturation: 94 °C, 30 seconds, hybridization: 51 °C, 50 seconds, elongation: 72 °C, 1 min or 2 min and 40 sec

25 cycles:
denaturation: 94 °C, 30 seconds
hybridization: 70 °C, 50 seconds
elongation: 72 °C, 1 min or 2 min and 40 sec

72 °C, 7 min
4 °C

The elongation time is 1 min for GAS antigens encoded by ORFs shorter than 2000 bp, and 2 min and 40 seconds for ORFs longer than 2000 bp. The amplifications are performed using a Gene Amp PCR system 9600 (Perkin Elmer).

To check the amplification results, 4 μl of each PCR product is loaded onto 1-1.5 agarose gel and the size of amplified fragments compared with DNA molecular weight standards (DNA markers III or IX, Roche). The PCR products are loaded on agarose gel and after electrophoresis the right size bands are excised from the gel. The DNA is purified from the agarose using the Gel Extraction Kit (Qiagen) following the instruction of the manufacturer. The final elution volume of the DNA is 50 μl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8). One μl of each purified DNA is loaded onto agarose gel to evaluate the yield.

(e) *Digestion of PCR fragments*

One-two μg of purified PCR products are double digested overnight at 37 °C with the appropriate restriction enzymes (60 units of each enzyme) using the appropriate restriction buffer in 100 μl final volume. The restriction enzymes and the digestion buffers are from New England Biolabs. After purification of the digested DNA (PCR purification Kit, Qiagen) and elution with 30 μl TE, 1 μl is subjected to agarose gel electrophoresis to evaluate the yield in comparison to titrated molecular weight standards (DNA markers III or IX, Roche).

(f) *Digestion of the cloning vectors (pET21b+ and pGEX-NNH)*

10 μg of plasmid is double digested with 100 units of each restriction enzyme in 400 μl reaction volume in the presence of appropriate buffer by overnight incubation at 37 °C. After electrophoresis on a 1% agarose gel, the band corresponding to the digested vector is purified from the gel using the Qiagen Qiaex II Gel Extraction Kit and the DNA was eluted with 50 μl TE. The DNA concentration is evaluated by measuring $OD_{260}$ of the sample.

(g) *Cloning of the PCR products*

Seventy five ng of the appropriately digested and purified vectors and the digested and purified fragments corresponding to each selected GAS antigen are ligated in final volumes of 10-20 µl with a molar ratio of 1:1 fragment/vector, using 400 units T4 DNA ligase (New England Biolabs) in the presence of the buffer supplied by the manufacturer. The reactions are incubated overnight at 16 °C. Transformation of *E coli* BL21 (Novagen) and *E coli* BL21-DE3 (Novagen) electrocompetent cells is performed using pGEX-NNH ligations and pET21b+ ligations respectively. The transformation procedure is as follows: 1-2 µl the ligation reaction is mixed with 50 µl of ice cold competent cells, then the cells are poured in a gene pulser 0.1 cm electrode cuvette (Biorad). After pulsing the cells in a MicroPulser electroporator (Biorad) following the manufacturer instructions the cells are suspended in 0.95 ml of SOC medium and incubated for 45 min at 37 °C under shaking. 100 and 900 µl of cell suspensions are plated on separate plates of agar LB 100 µg/ml Ampicillin and the plates are incubated overnight at 37 °C. The screening of the transformants is done by PCR: randomly chosen transformants are picked and suspended in 30 µl of PCR reaction mix containing the PCR buffer, the 4 dNTPs, 1,5 mM $MgCl_2$, Taq polymerase and appropriate forward and reverse oligonucleotide primers that are able to hibridize upstream and downstream from the polylinker of pET21b+ or pGEX-NNH vectors. After 30 cycles of PCR, 5 µl of the resulting products are run on agarose gel electrophoresis in order to select for positive clones from which the expected PCR band is obtained. PCR positive clones are chosen on the basis of the correct size of the PCR product, as evaluated by comparison with appropriate molecular weight markers (DNA markers III or IX, Roche).

2. Protein expression

PCR positive colonies are inoculated in 3 ml LB 100 µg/ml Ampicillin and grown at 37 °C overnight. 70 µl of the overnight culture is inoculated in 2 ml LB/Amp and grown at 37 °C until $OD_{600}$ of the pET clones reached the 0,4-0,8 value or until $OD_{600}$ of the pGEX clones reached the 0,8-1 value. Protein expression is then induced by adding 1 mM IPTG (Isopropil β-D thio-galacto-piranoside) to the mini-cultures. After 3 hours incubation at 37 °C the final $OD_{600}$ is checked and the cultures are cooled on ice. After centrifugation of 0.5 ml culture, the cell pellet is suspended in 50 µl of protein Loading Sample Buffer (60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% w/v Bromophenol Blue, 100 mM DTT) and incubated at 100 °C for 5 min. A volume of boiled sample corresponding to 0.1 $OD_{600}$ culture is analysed by SDS-PAGE and Coomassie Blue staining to verify the presence of induced protein band.

3. Purification of the recombinant proteins

Single colonies are inoculated in 25 ml LB 100 µg/ml Ampicillin and grown at 37 °C overnight. The overnight culture is inoculated in 500 ml LB/Amp and grown under shaking at 25 °C until $OD_{600}$ 0.4-0.7. Protein expression is then induced by adding 1 mM IPTG to the cultures. After 3.5 hours incubation at 25 °C the final $OD_{600}$ is checked and the cultures are cooled on ice. After centrifugation at 6000 rpm (JA10 rotor, Beckman), the cell pellet is processed for purification or frozen at –20° C.

(a) *Procedure for the purification of soluble His-tagged proteins from E.coli*

(1) Transfer the pellets from –20°C to ice bath and reconstitute with 10 ml 50 mM $NaHPO_4$ buffer, 300 mM NaCl, pH 8,0, pass in 40-50 ml centrifugation tubes and break the cells as per the following outline.

(2) Break the pellets in the French Press performing three passages with in-line washing.

(3) Centrifuge at about 30-40000 x g per 15-20 min. If possible use rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.)

(4) Equilibrate the Poly-Prep columns with 1 ml Fast Flow Chelating Sepharose resin with 50 mM phosphate buffer, 300 mM NaCl, pH 8,0.

(5) Store the centrifugation pellet at –20°C, and load the supernatant in the columns.

(6) Collect the flow through.

(7) Wash the columns with 10 ml (2 ml + 2 ml + 4 ml) 50 mM phosphate buffer, 300 mM NaCl, pH 8.0.

(8) Wash again with 10 ml 20 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8.0.

(9) Elute the proteins bound to the columns with 4.5 ml (1.5 ml + 1.5 ml + 1.5 ml) 250 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8,0 and collect the 3 corresponding fractions of ~1.5 ml each. Add to each tube 15 µl DTT 200 mM (final concentration 2 mM)

(10) Measure the protein concentration of the first two fractions with the Bradford method, collect a 10 µg aliquot of proteins from each sample and analyse by SDS-PAGE. (N.B.: should the sample be too diluted, load 21 µl + 7 µl loading buffer).

(11) Store the collected fractions at +4°C while waiting for the results of the SDS-PAGE analysis.

(12) For immunisation prepare 4-5 aliquots of 100 µg each in 0.5 ml in 40% glycerol. The dilution buffer is the above elution buffer, plus 2 mM DTT. Store the aliquots at –20°C until immunisation.

(b) *Purification of His-tagged proteins from Inclusion bodies*

Purifications are carried out essentially according the following protocol:

(1) Bacteria are collected from 500 ml cultures by centrifugation. If required store bacterial pellets at -20°C. For extraction, resuspend each bacterial pellet in 10 ml 50 mM TRIS-HCl buffer, pH 8,5 on an ice bath.

(2) Disrupt the resuspended bacteria with a French Press, performing two passages.

(3) Centrifuge at 35000 x g for 15 min and collect the pellets. Use a Beckman rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.).

(4) Dissolve the centrifugation pellets with 50 mM TRIS-HCl, 1 mM TCEP {Tris(2-carboxyethyl)-phosphine hydrochloride, Pierce} , 6M guanidium chloride, pH 8.5. Stir for ~ 10 min. with a magnetic bar.

(5) Centrifuge as described above, and collect the supernatant.

(6) Prepare an adequate number of Poly-Prep (Bio-Rad) columns containing 1 ml of Fast Flow Chelating Sepharose (Pharmacia) saturated with Nichel according to manufacturer recommendations..

Wash the columns twice with 5 ml of $H_20$ and equilibrate with 50 mM TRIS-HCl, 1 mM TCEP, 6M guanidinium chloride, pH 8.5.

(7) Load the supernatants from step 5 onto the columns, and wash with 5 ml of 50 mM TRIS-Hcl buffer, 1 mM TCEP, 6M urea, pH 8.5

(8) Wash the columns with 10 ml of 20 mM imidazole, 50 mM TRIS-HCl , 6M urea, 1 mM TCEP, pH 8.5. Collect and set aside the first 5 ml for possible further controls.

(9) Elute the proteins bound to the columns with 4.5 ml of a buffer containing 250 mM imidazole, 50 mM TRIS-HCl, 6M urea, 1 mM TCEP, pH 8.5. Add the elution buffer in three 1.5 ml aliquots, and collect the corresponding 3 fractions. Add to each fraction 15 µl DTT (final concentration 2 mM).

(10) Measure eluted protein concentration with the Bradford method, and analyse aliquots of ca 10 µg of protein by SDS-PAGE.

(11) Store proteins at -20°C in 40% (v/v) glycerol, 50 mM TRIS-HCl, 2M urea, 0.5 M arginine, 2 mM DTT, 0.3 mM TCEP, 83.3 mM imidazole, pH 8.5.

(c) *Procedure for the purification of GST-fusion proteins from E.coli*

(1) Transfer the bacterial pellets from –20°C to an ice bath and suspend with 7,5 ml PBS, pH 7,4 to which a mixture of protease inhibitors (CØMPLETE™ - Boehringer Mannheim, 1 tablet every 25 ml of buffer) has been added.

(2) Transfer to 40-50 ml centrifugation tubes and sonicate according to the following procedure:

a. Position the probe at about 0,5 cm from the bottom of the tube
b. Block the tube with the clamp
c. Dip the tube in an ice bath
d. Set the sonicator as follows: Timer → Hold, Duty Cycle → 55, Out. Control → 6.
e. perform 5 cycles of 10 impulses at a time lapse of 1 minute (i.e. one cycle = 10 impulses + ~45" hold; b. 10 impulses + ~45" hold; c. 10 impulses + ~45" hold; d. 10 impulses + ~45" hold; e. 10 impulses + ~45" hold).

(3) Centrifuge at about 30-40000 x g for 15-20 min. E.g.: use rotor Beckman JA 25.50 at 21000 rpm, for 15 min.

(4) Store the centrifugation pellets at –20°C, and load the supernatants on the chromatography columns, as follows

(5) Equilibrate the Poly-Prep (Bio-Rad) columns with 0,5 ml (≅1 ml suspension) of Glutathione-Sepharose 4B resin, wash with 2 ml (1 + 1) $H_2O$, and then with 10 ml (2 + 4 + 4) PBS, pH 7,4.

(6) Load the supernatants on the columns and discard the flow through.

(7) Wash the columns with 10 ml (2 + 4 + 4) PBS, pH 7.4.

(8) Elute the proteins bound to the columns with 4.5 ml of 50 mM TRIS buffer, 10 mM reduced glutathione, pH 8.0, adding 1.5 ml + 1.5 ml + 1.5 ml and collecting the respective 3 fractions of ~1.5 ml each.

(9) Measure the protein concentration of the first two fractions with the Bradford method, analyse a 10 µg aliquot of proteins from each sample by SDS-PAGE. (N.B.: if the sample is too diluted load 21 µl (+ 7 µl loading buffer).

(10) Store the collected fractions at +4°C while waiting for the results of the SDS-PAGE analysis.

(11) For each protein destined to the immunisation prepare 4-5 aliquots of 100 µg each in 0.5 ml of 40% glycerol. The dilution buffer is 50 mM TRIS.HCl, 2 mM DTT, pH 8.0. Store the aliquots at –20°C until immunisation.

4. Murine Model of Protection from GAS Infection

(a) *Immunization protocol*

Groups of 10 CD1 female mice aged between 6 and 7 weeks are immunized with two or more GAS antigens of the invention, (20 µg of each recombinant GAS antigen), suspended in 100 µl of suitable solution. Each group receives 3 doses at days 0, 21 and 45. Immunization is performed through intra-peritoneal injection of the protein with an equal volume of Complete Freund's Adjuvant (CFA) for the first dose and Incomplete Freund's Adjuvant (IFA) for the following two doses. In each immunization scheme negative and positive control groups are used.

For the negative control group, mice are immunized with *E. coli* proteins eluted from the purification columns following processing of total bacterial extract from a E. coli strain containing either the pET21b or the pGEX-NNH vector (thus expressing GST only) without any cloned GAS ORF (groups can be indicated as HisStop or GSTStop respectively).

For the positive control groups, mice are immunized with purified GAS M cloned from either GAS SF370 or GAS DSM 2071 strains (groups indicated as 192SF and 192DSM respectively).

Pooled sera from each group is collected before the first immunization and two weeks after the last one. Mice are infected with GAS about a week after.

Immunized mice are infected using a GAS strain different from that used for the cloning of the selected proteins. For example, the GAS strain can be DSM 2071 M23 type, obtainable from the German Collection of Microorganisms and Cell Cultures (DSMZ).

For infection experiments, DSM 2071 is grown at 37° C in THY broth until $OD_{600}$ 0.4. Bacteria are pelletted by centrifugation, washed once with PBS, suspended and diluted with PBS to obtain the appropriate concentration of bacteria/ml and administered to mice by intraperitoneal injection.

Between 50 and 100 bacteria are given to each mouse, as determined by plating aliquots of the bacterial suspension on 5 THY plates. Animals are observed daily and checked for survival.

5. Analysis of Immune Sera

(a) *Preparation of GAS total protein extracts*

Total protein extracts are prepared by incubating a bacterial culture grown to $OD_{600}$ 0.4-0.5 in Tris 50mM pH 6.8/mutanolysin (20 units/ml) for 2 hr at 37° C, followed by incubation for ten minutes on ice in 0.24 N NaOH and 0.96% β-mercaptoethanol. The extracted proteins are precipitated by addition of trichloroaceticacid, washed with ice-cold acetone and suspended in protein loading buffer.

(b) *Western blot analysis*

Aliquots of total protein extract mixed with SDS loading buffer (1x: 60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% Bromophenol Blue, 100 mM DTT) and boiled 5 minutes at 95° C, were loaded on a 12.5% SDS-PAGE precast gel (Biorad). The gel is run using a SDS-PAGE running buffer containing 250 mM TRIS, 2.5 mM Glycine and 0.1 %SDS. The gel is electroblotted onto nitrocellulose membrane at 200 mA for 60 minutes. The membrane is blocked for 60 minutes with PBS/0.05 % Tween-20 (Sigma), 10% skimmed milk powder and incubated O/N at 4° C with PBS/0.05 % Tween 20, 1% skimmed milk powder, with the appropriate dilution of the sera. After washing twice with PBS/0.05 % Tween, the membrane is incubated for 2 hours with peroxidase-conjugated secondary anti-mouse antibody (Amersham) diluted 1:4000. The nitrocellulose is washed three times for 10 minutes with PBS/0.05 % Tween and once with PBS and thereafter developed by Opti-4CN Substrate Kit (Biorad).

(c) *Preparation of Paraformaldehyde treated GAS cultures*

A bacterial culture grown to $OD_{600}$ 0.4-0.5 is washed once with PBS and concentrated four times in PBS/0.05 % Paraformaldehyde. Following 1 hr incubation at 37° C with shacking, the treated culture is kept overnight at 4° C and complete inactivation of bacteria is then controlled by plating aliquots on THY blood agar plates.

(d) *FACS analysis of Paraformaldehyde treated GAS coltures with mouse immune sera*

About $10^5$ Paraformaldehyde inactivated bacteria are washed with 200 µl of PBS in a 96 wells U bottom plate and centrifuged for 10 min. at 3000g, at 4°C. The supernatant is discarded and the bacteria are suspended in 20 µl of PBS-0.1%BSA. Eighty µl of either pre-immune or immune mouse sera diluted in PBS-0.1%BSA are added to the bacterial suspension to a final dilution of either 1:100, 1:250 or 1:500, and incubated on ice for 30 min. Bacteria are washed once by adding 100 µl of PBS-0.1%BSA, centrifuged for 10 min. at 3000g, 4°C, suspended in 200 µl of PBS-0.1%BSA, centrifuged again and suspended in 10 µl of Goat Anti-Mouse IgG, $F(ab')_2$ fragment specific-R-Phycoerythrin-conjugated (Jackson Immunoresearch Laboratories Inc., cat.N°115-116-072) in PBS-0.1%BSA to a final dilution of 1:100, and incubated on ice for 30 min. in the dark. Bacteria are washed once by adding 180 µl of PBS-0.1%BSA and centrifuged for 10 min. at 3000g, 4°C. The supernatant is discarded and the bacteria were suspended in 200 µl of PBS. Bacterial suspension is passed through a cytometric chamber of a FACS Calibur (Becton Dikinson, Mountain View, CA USA) and 10.000 events are acquired. Data are analysed using Cell Quest Software (Becton Dikinson, Mountain View, CA USA) by drawing a morphological dot plot (using forward and side scatter parameters) on bacterial signals. An histogram plot is then created on FL2 intensity of fluorescence log scale recalling the morphological region of bacteria.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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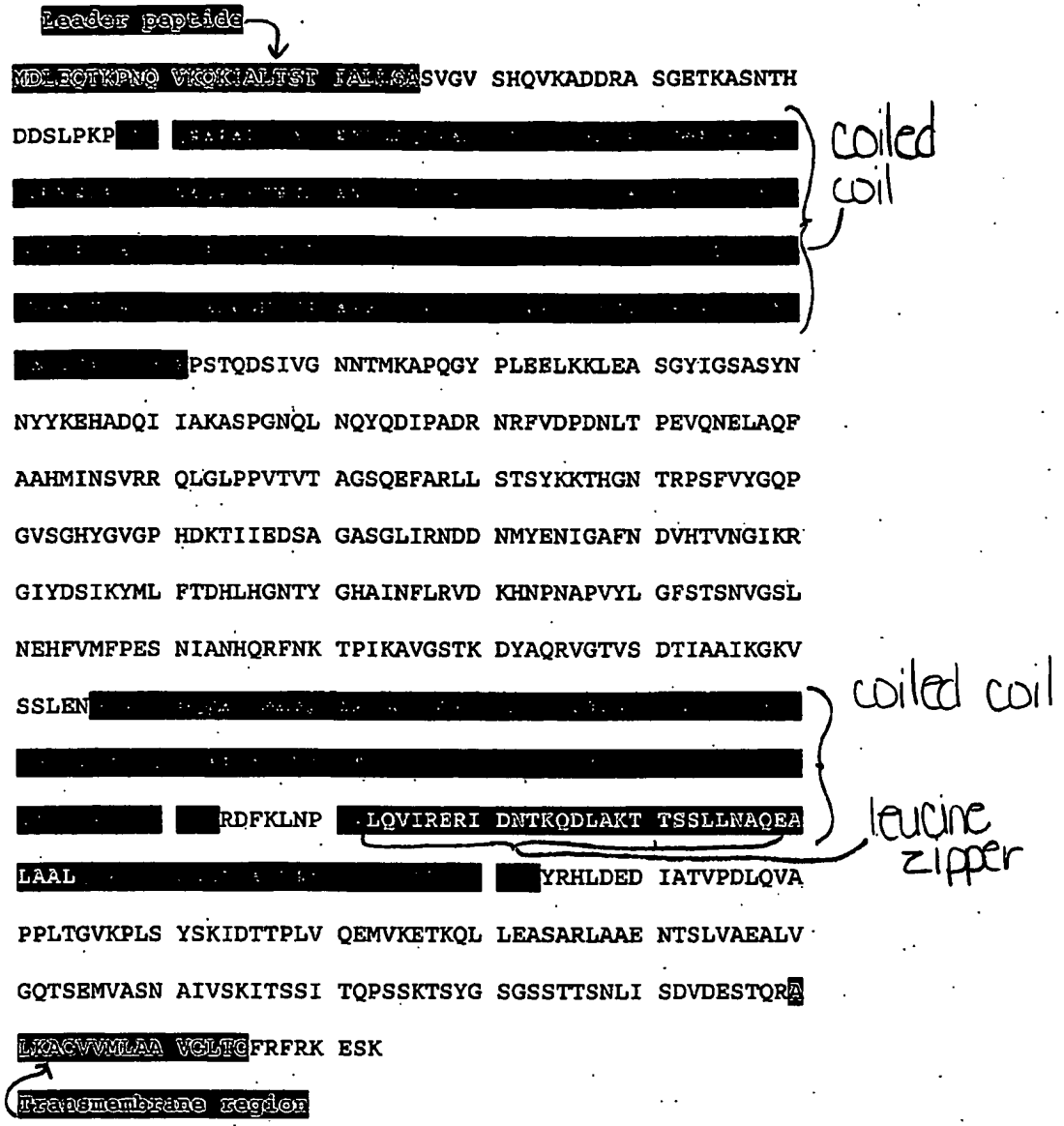
# FIGURE 1: Annotation of GAS 40





# FIGURE 2 : Schematic of GAS40: putative surface exclusion protein prgA (873aa)



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